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ROLE OF PLANT COVER IN THE PERFORMANCES OF EXTENSIVE GREEN ROOFS IN SUB MEDITERRANEAN ENVIRONMENT

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ABSTRACT

Green roofs provide many benefits to the urban environment, but an adequate plant cover is crucial in order to achieve optimal performances from these infrastructures. Plant cover improves the roof insulation in winter and reduces summer temperatures by shading and transpiration.

Succulent species, such as *Sedum* species, are widely used on green roofs, thanks to their drought resistance due to CAM metabolism, as well as to the reduced maintenance they need. CAM metabolism, however, without diurnal transpiration, is responsible for the poor cooling performances in summer. In addition, many succulent species have slow colonization rates and/or are subject to freezing injury during the winter. A reduced plant cover has direct negative consequences both on the cooling by shading and on the winter insulation.

We investigated how to create green roofs with a good plant cover, optimal summer and winter thermal performances and reduced management needs. Primarily, we compared, thought indoor experiments, the water losses, the net CO_2 assimilation rates and the cooling performances of two CAM-facultative, three C3 and one C4 species, under well-watered and drought conditions. Our results confirmed that C3 and C4 tested species continue to transpire also when *Sedum* species adopt the CAM metabolism, suppressing the diurnal transpiration. C4 metabolism allows interesting transpiration performances, even under very droughty conditions. This has a positive effect on the substrate temperatures, but, at the same time, a good canopy cover is necessary. It is fundamental for shadow effect in summer, but also to increase the minimal temperatures of substrate in winter.

We also monitored the way of growth of six native perennial species, cultivated on an experimental green roof under a low-input management. The six species, thanks to their different growth behaviors, could provide the good coverage necessary to guarantee the main green roof benefits.

Keywords: Green roof; Substrate Moisture Content (SMC); Relative Water Content (RWC); C3 species; C4 species; CAM-facultative species; cooling effect; water stress; winter insulation performance; plant cover; *Achillea millefolium* L.; *Acinos alpinus* (L.) Moench; *Bromus erectus* Huds; *Cynodon dactylon* (L.) Pers.; *Festuca ovina* L.; *Hieracium pilosella* L.; *Lotus corniculatus* L.; *Salvia officinalis* L.; *Sanguisorba minor* Scop.; *Sedum kamtschaticum* Fisch.; *Sedum lydium* Boiss; *Thymus serpyllum* L.

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1. GENERAL INTRODUCTION

According to Huong and Pathirana (2011), in 2008 for the first time, more than half of the world's population was living in cities. The biggest increase in population in urban areas was due to real population growth, to migration from rural areas to the cities and transformation of rural settlements into cities (Huong and Pathirana, 2011). The result is an uncontrolled urban sprawl with increasing human settlements, industrial growth and infrastructure development (UN, 2006). The increase in artificial surfaces due to urbanization causes an increase in flooding frequency due to poor infiltration and reduction of flow resistance and to hydrological and hydroclimatological changes (WMO/GWP, 2008). The absence of green spaces decreases the evapotranspiration and hence the increase of the "urban heat island" (UHI) effect in the urban areas. In fact, the radiative properties of the urban environment are distinctly different, because urban areas absorb more radiation due to the nature of the urban canopy. These changes in surface heat budget provide the particular atmospheric conditions over urbanized areas that caused the UHI. (Shepherd *et al.*, 2002; Pathirana *et al.*, 2013).

These negative effects can be reduced by introducing plant cover on the unutilized surfaces, like the rooftop of buildings, as a valuable strategy to make buildings more sustainable (Vijayaraghavan, 2016).

Green roofs are green infrastructures consisting of vegetation, growth medium layer and other specific layers, built over the rooftops of buildings. The role of each component (vegetation, growth medium, filter fabric, drainage material, root barrier and insulation) is well defined in engineered green roof system and types of each green roof component depends on the geographic location and the climatic conditions (Vijayaraghavan, 2016).

Green roofs are classified into intensive, semi-intensive and extensive green roofs (Snodgrass and McIntyre, 2010; Vijayaraghavan, 2016). Intensive green roofs (IGR) are characterized by thick substrate layer (more than 20 cm), wide variety of plants, high maintenance, high capital cost and greater weight. Thanks to high substrate depth, the plant selection can include shrubs and small trees (Berardi *et al.*, 2014). Therefore, typically it require high maintenance in the form of fertilizing, weeding and watering. On the other hand, extensive green roofs (EGR) are characterized by thin substrate layer (about 15 cm), low capital cost, low weight and very low maintenance. (Because of the thin substrate layer, it is possible to plant on EGR limited types of plants including grasses, moss and in particular succulent species (Berardi *et al.*, 2014). EGRs are commonly used in situations where no additional structural support is desired. Semi-intensive green roofs accommodate small herbaceous plants, small grasses and small shrubs due to moderately thick substrate layer. These roofs require frequent maintenance as well as sustained high capital costs. Of the three types, EGRs are most common around the world due to building weight restrictions, costs and maintenance (Vijayaraghavan, 2016). However, there is no roof without maintenance, green or traditional (Snodgrass and McIntyre, 2010). The maintenance required will depend on the outcome desired by the client, for this reason, it is preferable to plan the maintenance schedule with the client during the design process. The maintenance of green roofs is considered as one of the greatest barriers to their installation. For example, usually, for extensive green roofs with *Sedum* mix it is recommending weeding three times a year and application of fertilizer once a year. Semi-intensive roofs and intensive green roofs, designed with aesthetic appeal or the functionality of a roof garden, will necessarily require a more intensive maintenance regime, as in most gardens.
A proper maintenance schedule often makes the difference between a thriving green roof and failed one. (Snodgrass and McIntyre, 2010).

Modern green roofs started to be designed, developed and marketed on a larger scale at the turn of the 20th century in Germany (Oberndorfer *et al.*, 2007). At first, vegetation was installed on roofs as fire-retardant (Köhler 2003) or to mitigate the damaging physical effects of solar radiation on the roof structure (Oberndorfer *et al.*, 2007). In the 1970s, growing environmental concern, especially in urban areas, created opportunities to introduce progressive environmental thought in Germany and then in neighboring European countries and in other parts of the world. It is estimated that the green roof coverage in Germany alone increases by approximately 13.5 million m^2 per year (Oberndorfer *et al.*, 2007); whereby 10 % of its buildings utilize green roof technology (Vijayaraghavan, 2016).

Countries like USA, Canada, Australia, Singapore and Japan are encouraging the construction of new roofs or to retrofit old buildings so green roofs can be added in the future. For example, in Toronto (Canada), the law requires that on all new buildings with an area equal to or greater than 2000 m^2, there is a green roof on 20 - 60% of the roof surface (Chen, 2013; Vijayaraghavan, 2016). Similarly, Tokyo (Japan) impose that private buildings larger than 1000 m^2 and public buildings larger than 250 m^2 must green 20 % of the rooftop or pay an annual penalty of USD 2000 (Chen, 2013). Regarding to the USA, in Portland it is required that green roofs cover at least 70 % of the roof (Townshend, 2007). About 2 acres (0.81 ha) of green roofs were build in Portland (USA) in 2005 an approximately another 2 acres were committed (Townshend, 2007). Also the government of Hong Kong, encourages the construction of green roofs (Zhang *et al*., 2012).

Green roofs present numerous economic and social benefits in addition to more obvious environmental advantages such as storm–water management, decreased energy consumption of buildings, improved water and air quality, decreased noise pollution, extended roof life, reduced UHI and increased green space in urban environments (Speak *et al*., 2013; Eksi *et al*., 2017). In the order to increase knowledge about these benefits, the pace and number of publications in the field of green roof increased significantly all over the world compared to early 2000 (Vijayaraghavan, 2016). However, each country with different climatic conditions and building characteristics needs local research to identify the best components (vegetation, substrate or stratification) for successful establishment of green roofs (Del Barrio**,** 1998; Vijayaraghavan, 2016). These aspects acted as driving force behind the increase in green roof research. For example, Graceson *et al.* (2014) and Vijayaraghavan and Raja (2014) tested different types of waste material (like crushed bricks and composted green waste) as green roof substrate (Graceson *et al*., 2014; Vijayaraghavan and Raja, 2014). On the other hand, Razzaghmanesh *et al.* (2014) examined several indigenous Australian ground covers and grass species and identified that *Carpobrotus rossii* tolerated hot and dry conditions of South Australia with 100 % survival rate and maximum growth (Razzaghmanesh *et al.*, 2014; Vijayaraghavan, 2016). Closer to the Mediterranean area, in Spain, Vestrella *et al.* (2015) tested two simulated green roofs planted with a variety of species (divided into three groups according to growth form) and subjected to both minimal irrigation and no irrigation treatment. This study demonstrated that it is possible to obtain good results from a Mediterranean green roof but an appropriate combination of vegetal species with different structures and development, was necessary.

1.1 Benefits and advantages of green roofs

Despite the various potential benefits provided by the green roofs have been known since several years, the focus of most green roof developers and designers has been mainly addressed to satisfy the aesthetical benefits achievable from these structures (Berndtsson et al., 2010). Important environmental benefits, could be also achieved, but the majority of commercial green roofs are not designed to meet them (Vijayaraghavan and Joshi, 2014). According to Vijayaraghavan (2016), this is caused generally by premature introduction of products into the market and lack of researches carried out on the design of green roofs, in particular on the choose of plant species to use and how these choices could influence the performances of green roofs themselves.

Below, the state of the art, taken from recent literature on the subject, is presented. Literature concerning the thermal performances of green roofs (mitigation of the urban heat island effect, winter insulation), subject to which three experiments of the thesis are dedicated, is particularly in-depth. A less extensive survey, concerning other environmental benefits that green roofs can provide, is also presented. (Vestrella *et al*., 2015).

1.1.1 Thermal performances and energy saving

As confirmed by the recent work of Eksi *et al.* (2017), in the last ten years, numerous studies have shown that green roofs can lower roof surface, substrate, and waterproof membrane temperatures, as well as moderate heat flux into and out of a building and save energy costs for heating and cooling (Saadatian *et al*., 2013). Getter *et al.* (2011) found that a shallow extensive green roof reduced heat flow through the building by an average of 13 % in winter and 167 % during summer. Meanwhile, summer temperature values below the substrate were 20 °C cooler than the traditional roof without the green roof protection (Getter *et al*., 2011). The reductions of temperature fluctuations of roof membranes prolong their lifespan due to less expansion and contraction (Oberndorfer *et al.*, 2007). It is possible to act on the thermal properties of a roof in three ways: thought the substrate layer, the plant canopy shades the roof surface and evapotranspiration of plant and substrate layers (Ouldboukhitine *et al.*, 2012; Saadatian *et al.*, 2013). The influence of each way depends on day to day weather conditions such as solar radiation, air temperature, precipitation and thus substrate moisture, and snow cover (Eksi *et al.*, 2017). Other important factors to consider are depth and composition of the growing layer, plant species and the green roof management (irrigation and fertilization). Some studies have shown that substrate depth and typology influence heat flux into or out of the building (Wong *et al.*, 2003; Sailor, 2008). Solar radiation can heat the entire substrate volume much faster when the depth is shallow (Jim and Peng, 2012). On the other hands, Lundholm *et al.* (2014) reported that the substrate depth from 7.5 cm to 15 cm had no impact in lowering net heat loss (Lundholm *et al.*, 2014). This contradiction could be due to substrate composition and compaction. Sailor and Hagos (2011) concluded that substrates consisting of heat-expanded slate showed a thermal conductivity that was two to three times more than substrates that incorporated a silica-based aggregate (Sailor and Hagos, 2011). Thus, besides the substrate typology, also the density, particle size and air pockets in the substrate influence thermal conductivity of green roofs (Saadatian *et al.*, 2013). As density increases, thermal conductivity and heat flux will also increase (Castleton *et al.*, 2010). The aging of the substrate increases the compactness, consequently thermal conductivity is likely to increase and thus the substrate’s ability to act as an insulator will decrease. Thermal conductivities may also decrease by 30 – 40 % than original values due to compaction (Sailor and Hagos, 2011).

Transpiration activity of plants influences the temperatures. Thus, in theory, plant species with greater biomass and higher transpiration rates should provide a greater cooling effect (Eksi *et al.*, 2017). This has been shown to be true (Tabares-Velasco and Srebric, 2009; Fioretti *et al.*, 2010), but, in other situations this has been shown not to be true (Franzaring *et al.*, 2016; MacIvor *et al.*, 2016). Leaf area index (LAI), stomatal resistance, height, coverage and albedo are five characteristics that influence the plants thermal performances (Saadatian *et al.*, 2013). Plants increase the cooling during summer, but plant type can also influence the depth of snow cover during winter that has an effect on heat flux in the winter (Lundholm *et al.*, 2014; Buckland-Nicks *et. al.*, 2016). Although, plant transpiration rates has an effect on cooling, plant coverage of the surface may be more important (Eksi *et al.*, 2017). Shade provided by higher LAI will influence the overall albedo of the roof (Sailor, 2008) and decrease solar radiation that reaches the substrate surface (Yaghoobian and Srebric, 2015; Eksi *et al.*, 2017). The surface cooling potential due to broadleaf was confirmed by Blanusa *et al.* (2013) that showed a significant reduction of surface temperature up to 12 °C. Furthermore, Bowler *et al.* (2010) measured the daily gain in heat on roofs containing shrubs, trees, turf, and bare soil (Bowler *et al.*, 2010). All daily gain in heat of these roofs was significantly higher than the conventional roof. Analyzing more in detail the influence of growth form of species used and the structure of the plant cover, Franzaring *et al.* (2016) reported that monocultures of *Phedimus floriferus* (a succulent species with wide and water-filled leaves) and *Lotus corniculatus* (a common flowering plant with small but wide compound leaves (made up 3 leaflets) provided better cooling than the erect species *Dianthus carthusianorum* and the grass *Koeleria glauca* (two erect species characterized by narrow leaves). These results are probably due to the ability of the first two species to shadow the substrate more efficiently than the upright, blade-leaved species. In addition to the morphological features of different plant species, Lundholm *et al.* (2015), testing 21 herbaceous species with different growth form, found that substrate cooling was strongly related to canopy density.

1.1.2 Other environmental benefits

The thermal performance of green roofs is ones of the most studied green roof’s benefits due to its high influence on UHI effect thus, also for this reason, it is the principal aspect of green roof considered in this thesis. However, green roofs offer additional economic and ecological services, such as storm-water management, reduction of noise and air pollution, improving roof membrane longevity, providing space for urban food production, providing habitat for wildlife, and improving human health (Eksi *et al.*, 2017).

STORM-WATER MANAGEMENT. From a hydraulic perspective, urban areas are dominated by hard, waterproof surfaces that contribute to heavy runoff, which can overburden existing storm-water management facilities and cause combined sewage overflow into lakes and rivers (Oberndorfer *et al.*, 2007). In addition to increase the flooding, erosion, and sedimentation, urban rainwater is also charged with pollutants such as pesticides and petroleum residues, which pollute wildlife habitats and drinking supplies (Moran *et al.*, 2005). Several studies have shown that green roofs have significant effects on retaining rainfall volumes (Garofalo *et al.*, 2016), delaying the peak flow rate (Sailor, 2008; Garofalo *et al.*, 2016) and reducing the runoff volume discharged into the combined sewer systems (CSSs) (Jim and Peng, 2012; Garofalo *et al.*, 2016). Green roofs are ideal for urban storm-water management because they make use of existing roof space to store water during rainfall events, delaying runoff ~~until~~ after peak rainfall and returning precipitation to the land. DeNardo *et al.* (2005) and VanWoert *et al.* (2005) showed that green roofs could lead to 60 % runoff mitigation for extensive green roofs and up to 90 % for intensive green roofs. Moran *et al.* (2005), in Oregon, observed that rainfall retention from specific green roofs was 66 % to 69 % for roofs with more than 10 cm of substrate.

Factors which influence green roof water retention capacity and runoff dynamics depend on weather conditions (air temperature, wind conditions, humidity and characteristics of rain event) and on the green roof characteristics (Berardi *et al.*, 2014). For example the number of layers and type of materials, soil thickness, soil type, roof geometry, roof position (e.g. shadowed or not, faced direction), roof age but in particular the vegetation cover and type of vegetation.

In general, green roofs can reduce annual total building runoff by as much as 60 % to 79 % (Köhler *et al.*, 2002), and estimates based on 10 % green-roof coverage suggest that they can reduce overall regional runoff by about 2.7 % (Mentens *et al.* 2005).

WATER QUALITY. The role of green roofs in storm-water retention is well understood, but some research demonstrates that green-roof runoff includes increased levels of nitrogen and phosphorus due to leaching from the substrate (Dunnett and Kingsbury, 2004; Moran *et al.*, 2005). Organic matter, nutrients, and contaminants in the growing medium or roof membranes can cause discharged water to be a new source of surface-water pollution. Reducing the fertilization of green roof vegetation should also improve runoff water quality but may reduce plant growth and coverage. Selecting plants that optimize the uptake of nutrients and contaminants may help to reduce pollutants in runoff while promoting plant survival (Oberndorfer *et al.*, 2007).

AIR QUALITY. Vegetation growing on rooftops partially substitutes the vegetation demolished during construction. Green roofs, despite the low plant biomass they host, could be an urban carbon sink. It is well known, futhermore, that urban vegetation is able to trap airborne particulates and to take up other contaminants such as nitrogen oxides. The potential benefits of roof greening for reducing the air pollution of NOx, SO_2 and PM10 are documented by Speak *et al.* (2014). Similarly, in Singapore, Tan and Sia (2005) reported that the contribution of green roofs on air pollutions reducing (in particular, SO_2) was around up to 37 % pollution removal.

Another important aspect to consider is the indirect reduction in pollution levels due to the energy saving of building and UHI reduction.

As UHI increases radiant temperature in the urban contest, thus, the effectiveness of green roofs in reducing the heat island will indirectly result in reducing energy consumption necessary for cooling loads of buildings (Berardi *et al.*, 2104). Many studies evaluated how the decrease of through green roofs would reduce the level of pollution indirectly (Berardi *et al.*, 2014).

SOUND INSULATION AND NOISE REDUCTION. Green roofs have often been proposed for their ability to sound absorption and noise insulation (Connelly and Hodgson, 2008; Van Renterghem and Botteldooren, 2011; Berardi *et al.*, 2014). Considering the transmission loss (TL), the number of sound decibels that are stopped by traditional roof (the reference roof) and two green roofs with two different depths of substrate (7.5 cm and 15 cm), Connelly and Hodgson (2008) revealed that the increase of TL through the green roof 7.5 cm depth at different frequencies was less consistent than through the green roof 15 cm depth. Also, it was demonstrate that deep green roof increased the TL between 5 dB and 13 dB at low and mid frequency bands (50–2000 Hz) and of less than 6 dB at higher frequencies. In addition, it is demonstrated that, on an urban scale, the green roofs decrease the noises at street level in urban areas thanks to the high absorption coefficient of the vegetation layer (Yang *et al.*, 2012; Berardi *et al.*, 2014). Obviously, this benefit is more evident in low green roofs because they are exposed to the direct urban noise.

IMPROVING ROOF MEMBRANE LONGEVITY. Waterproofing membranes on conventional roofs deteriorate rapidly because of the solar irradiation, in particular due to ultraviolet (UV) light. Also, membranes are consequently more easily damaged by widely fluctuating roof temperatures. By physically protecting against UV light and reducing temperature fluctuations, green roofs extend the life of the roof's waterproofing membrane and improve building energy conservation. Temperature stabilization of the waterproofing membranes by green-roof coverage may extend their useful life by more than 20 years (Oberndorfer *et al.*, 2007).

CONTRIBUTION TO URBAN BIODIVERSITY - GREEN ROOFS AS HABITATS. The role of biodiversity in living-roof performances has been little investigated, but the few studies made until now show promising results for the contribution of green roofs to biodiversity and habitat conservation. Specific studies have documented plant, lichens, invertebrate and avian communities on a variety of living-roof types in several countries (Coffman and Davis, 2005; Brenneisen, 2006; Kadas, 2006; Köhler, 2006; Orsini *et al.*, 2014). These findings have mobilized local and national conservation organizations to promote green-roof habitats, particularly in Switzerland and the United Kingdom. Furthermore, these results have encouraged discussion of green roof design strategies to maximize biodiversity (Brenneisen, 2006).

Green roofs could be inhabited by various insects, including beetles, ants, flies, bees, spiders, and leafhoppers (Coffman and Davis 2005), in particular, also rare and uncommon species of beetles and spiders (Brenneisen, 2006; Grant, 2006; Kadas, 2006). Species richness in spider and beetle populations on green roofs is positively correlated with plant species diversity and topographic variability (Gedge and Kadas, 2004). In addition, Kadas (2006) concluded that the potential of these particular artificial habitats is vast because he observed that at least 10 % of invertebrate housed were nationally rare or scarce (in UK).

Green roofs have also been used by nesting birds and native avian communities (Baumann 2006). Rare plants and lichens often establish spontaneously on older roofs as well (Brenneisen, 2006, Köhler, 2006). Despite the limited size, the green roofs has implications as habitats for the biodiversity and landscape properties of areas in which green roofs are installed (Köhler, 2006).

Living roofs, as substitutes for natural and semi-natural environments absent in cities, also provide aesthetic and psychological benefits for people in urban areas. Even when green roofs are only accessible as visual relief, the benefits may include relaxation and restoration (Hartig *et al*. 1991), which can improve human health. Recently, the concept of green roof has been associated to the enhancement of urban agriculture: food production can provide economic and educational benefits to urban dwellers (Oberndorfer *et al*., 2007; Orsini *et al*., 2014).

1.2 The role of the vegetation layer

According to the previous literature review, the vegetation layer has an important role on many benefits provided by the green roofs, depending on the morphological-structural characteristics, ecological and physiological adaptations of the used species. It is thus important to determine which plant species are more suitable for these surfaces, in order to obtain some environmental benefits from the vegetation growing under so particular and limiting environmental conditions (Butler and Orians, 2011). The main stress factors, especially in Mediterranean and sub-Mediterranean areas, are summer water deficit (Carter and Butler, 2008) and high temperatures (Martin and Hinckley, 2007)~~.~~

The main metabolic ways (CAM and CAM-facultative, C3 and C4) of the plant species represent different evolutionary-adaptative responses to the water use. Different advantages/disadvantages come from these physio-ecological adaptations (Herrera, 2009). They deserve particular consideration in function of a well targeted and aware utilization of species, belonging to these physio-ecological groups, for the vegetation layer of green roofs.

1.2.1 CAM and CAM-facultative species

CAM metabolism is a form of CO_2 assimilation that helps to extend the life of plant under drought conditions by reducing daytime transpiration. CAM plants CO_2 uptake occurs during the night, when stomata open; CO_2 is combined with phosphoenolpyruvate (PEP) through the action of PEP-carboxylase (PEPC) to yield oxaloacetate, which is reduced to malate. Malate is transported into the vacuole following the actively transported protons and malic acid accumulates during the night. Nocturnal acid accumulation and nocturnal stomatal aperture are the main diagnostic features of CAM. During the day, malate is decarboxylated in the cytoplasm, providing ribulose-1,5-bisphoshate carboxylase/oxygenase (Rubisco) with one of its substrates during C3 photosynthesis. The net outcome of the functioning of CAM is that CO_2 is fixed with significant water saving, relative to C3 photosynthesis, and the water-use efficiency. This means that water-use efficiency of CAM species increases, if compared to a C3 plant.

Three different types of CAM metabolism are known: 1) CAM-obligate, characterized by high nocturnal acid accumulation (ΔH^+) and CO_2 fixation; 2) CAM-facultative, also known as C3-CAM, with a C3 form of CO_2 fixation in the non-induced state, and small nocturnal CO_2 fixation and ΔH^+ in the induced state; 3) CAM-cycling, with daytime CO_2 fixation and ΔH^+, but no nocturnal stomatal aperture or with small ΔH^+ and stomatal closure during the entire day and night in severely stressed plants (Herrera, 2009).

CAM-facultative and CAM-cycling species typically grow in semi-arid regions, on rocks, in general, in habitats where water deficit is frequent. It is widely accepted that CAM is an adaptive mechanism which optimizes water use under conditions of deficient supply. In CAM-facultative species, CAM metabolism may be induced by factors such as drought (Borland and Griffiths, 1990; Herrera *et al.*, 1991), salinity (Winter and von Willert, 1972), photoperiod (Brulfert *et al.*, 1988), high photosynthetic photon flux (PPF) (Maxwell, 2002), nitrogen deficiency (Ota, 1988) and phosphorus deficiency (Paul and Cockburn, 1990).
Briefly, CAM-facultative species have the capacity to use C3 metabolism under well-watered conditions. When the availability of water begins to run out, they close stomata during the day to conserve water, and open stomata at night, to extract carbon dioxide when temperatures are cooler. This behavior helps extend the life of the plant under drought conditions and allows higher transpiration and biomass production than obligate CAM species. This is an important aspect to consider for their use on green roofs.

Succulent CAM species are the most popular types of plants used for extensive green roofs, because of their small size, reduced growth rate, short root structure, reduced maintenance, and their ability to limit transpiration and store water (Rowe *et al.*, 2012; Berardi *et al.*, 2014). They show the typical strategies of the stress-tolerant species (Grime, 1979). It is well know that *Sedum album*, *Sedum acre*, *Sedum kamtschaticum* **ssp.** *ellacombianum*, *Sedum pulchellum*, *Sedum reflexum*, and *Sedum spurium* survived around 80 days (or more) of drought (Rowell *et al.*, 2011) and several species of *Sedum* survived and maintained active photosynthetic metabolism even after four months without water (Rowell *et al.*, 2011). However, low transpiration activity, in particular under drought conditions, has a negative influence on the surface temperatures of roofs as well as on the mitigation of UHI effect (Eksi *et al.*, 2017). In addition to the low transpiration activity, the succulents species are not always suitable for application on the green roof, as they have slow colonization rates and are subject to freezing injury during the winter season (Benvenuti and Bacci, 2010) and this reduce their leaves' shading effect. The limited shading effect makes these species unable to avoid convective heat transfer under their usually small and narrow leaves; consequently, they show a low thermal resistance value (Berardi *et al.*, 2014).

1.2.2 C3 and C4 species.

C3 photosynthesis is the major of the three metabolic pathways for carbon fixation by plants. About 85 % of the plant species are C3, while only 4 % are C4. (Ghannoum, 2009) The C3 photosynthesis uses the enzyme RuBisCO, in relatively inefficient conditions, to fix CO_2 from the air and obtain the 3-carbon organic intermediate molecule 3-phosphoglycerate. C3 species possess a specific leaf structure, and are not adapted to non-optimal environmental conditions. The C3 photosynthesis, in fact, works well in relatively cool and wet environments. C3 plants lose 97 % of the water taken up through their roots to transpiration (Raven and Edwards, 2001) and water use efficiency (assimilation rate/transpiration rate) is 3-10 times lower in C3 than in CAM plants (Kluge and Ting, 1978).

As far as the utilization of C3 species for the green-roofs plant cover is concerned, plants with high transpiration rate play an important role on the rainfall retention and storage capacity of green roofs during storm events (Voyde *et al*. 2010). Berghage *et al*. (2007) calculated that plants provide up to 40 % of the total storm-water retention response.
Italian and Mediterranean floras are rich in native C3 herbaceous perennial species, adapted to summer drought stress. Benvenuti and Bacci (2010) used wild Mediterranean species, well adapted to summer drought stress as an alternative to the *Sedum* species, to sustain biodiversity, entomofauna and the entomophilous pollination.

Results obtained by Blanusa *et al.*, (2013) and Vaz Monteiro *et al.*, *(*2017) showed high influence of transpiration activity on the cooling effect during the drought season. They used a range of contrasting plant types, succulent and non-succulent species (commercial *Sedum* mix, *Stachys byzantina*, *Bergenia cordifolia* and *Hedera hibernica*). In addition, these two studies demonstrated that, despite the more frequent irrigation required by non-succulent species, it is possible to take advantage from other water-saving strategies of xerophilous species with hairy and narrow leaves.

The characteristic of C4 photosynthesis is the operation of a CO_2-concentrating mechanism in the leaves, which serves to saturate photosynthesis and suppress photorespiration in normal air (Ghannoum, 2009). The photosynthesis process of C4 plants is divided between mesophyll and bundle sheath cells. The first step occurs in the mesophyll and involve the hydration of CO_2 into bicarbonate, which reacts with phosphoenolpyruvate (PEP) with the aid of PEP carboxylase (PEPC) to produce oxaloacetate. Oxaloacetate is converted into other C4 acids (malate, aspartate or alanine) which diffuse into the bundle sheath cells where they are decarboxylated, releasing CO_2 for fixation by Rubisco and the rest of the C3 cycle.
Plants with C4 photosynthesis increase water use efficiency and suppress photorespiration during drought stress; thus C4 plants are often more competitive than C3 plants in drought environments (Edwards and Ku, 1987) and in hot sunny environments. As CAM pathway, C4 has evolved independently over two dozen times, which suggests it may give plant species in hot climates a significant evolutionary advantage (Guralnick et al., 2008).

Although C4 species make a significant contribution to the global carbon budget and their importance to future global food security increases because of the global warming (Brown, 1999; Pingali, 2001; Ghannoum, 2009), the response of C4 photosynthesis to water stress has been less well analyzed than C3 ones (Ghannoum, 2009). Several studies (Hura *et al*, 2007; Ghannoum *et al.*, 2008) compared the behaviors of C3 and C4 metabolism under drought conditions, in particular, Hura *et al.* (2007) observed that *Zea mays*, a C4 species, probably acclimatized more effectively to soil drought through the development of effective mechanisms for utilizing excitation energy in the photosynthetic conversion of light, accompanied by the mechanism protecting the photosynthetic apparatus against the excess of this energy.
Staats and Klett (1995) found that *S. acre* required less irrigation to maintain a pleasing leaf compared with C4 plants, but no specific research has been conducted on the use of C4 species on the green roofs, despite the efficient use of water of these species.

OBJECTIVES OF THESIS

Starting from the review on green roofs made by Oberndorfer (2007), an increasing number of researches and reviews (Benvenuti, 2014; Berardi *et al.*, 2014; Blanusa *et al.*, 2014) began to pay a growing attention to environmental performance and the multifunctional benefits that green roofs can provide.

In the early 2000s (VanWoert *et al.*, 2005; Mentens *et al.*, 2006) the attention to the green roofs was aimed at their function of storm water management (limiting the runoff and increasing the water retention), regardless of the role played by the vegetation cover to optimize this function. Around the years 2010 (Starry, 2013) the efficiency of plant cover is questioned. The school of Reading, linked to the activity of some important plant ecologists (Grime *et al.*, 1988) marks an important stage by applying basic knowledge previously acquired on a large number of species of British flora, to the realization of experimental green roofs. Plant ecologists and agronomists from the Mediterranean area (Benvenuti and Bacci, 2010; Caneva *et al.*, 2013; Benvenuti, 2014) propose lists of species belonging to the respective flora, or made experiments with species taken from these lists, for the realization of green roofs better integrated with the surrounding environmental context.

In the UK, Blanusa (2013) carried out one of the first studies that investigated on the role of plants on the performances of green roof. The different ways and strategies used by the species to grow and spread in the space, despite the environmental limits, are ecophysiological knowledge fundamental to optimize the relationships between plant species selection and green roof efficiency.

The European Commission has developed a Green Infrastructure Strategy (COM 2013). This strategy promotes the use of green infrastructures (including the green roofs) in order to provide cost-effective alternatives to traditional 'grey' infrastructure and offer many benefits for both citizens and biodiversity ~~or~~ to restore the degraded ecosystems (EU biodiversity strategy to 2020; Action 6: Restore ecosystems, maintain their services and promote the use of green infrastructures).

This PhD thesis aims to offer a contribution of knowledge on the use of alternative species (C3 and C4) on the green roofs through the results of a series of experiments performed in greenhouses and in open environments.

In particular, this study aims to answer some specific questions:

- What advantages and disadvantages can the different transpiration activities and different strategies of water use of C3 species *(Bromus erectus* Huds., Gramineae; *Lotus corniculatus* L., Leguminosae; *Salvia officinalis* L., Lamiaceae), C4 (*Cynodon dactylon* (L.) Pers., Gramineae) and CAM-facultative (*Sedum lydium* Boiss and *Sedum kamtschaticum* Fisch., Crassulaceae) provide to the cooling effect of green roofs during the summer?

- What are the winter insulation capacities of the same species?

- Which species of Italian spontaneous flora can be proposed for the realization of green roofs? Considering the following species: *Festuca ovina* L., Gramineae; *Thymus serpyllum* L., Lamiaceae; *Hieracium pilosella* L., Asteraceae; *Acinos alpinus* (L.) Moench, Lamiaceae; *Sanguisorba minor* Scop. Rosaceae and *Achillea millefolium* L., Asteraceae), which morphological parameters and which traits (growth behavior, habitus, spreading and seeding capacity) are of particular interest in order to obtain a good plant cover?

2. **THERMAL PERFORMANCES OF PLANT SPECIES**

2.1 FIRST, SECOND and THIRD EXPERIMENTS: The green roof's cooling performances: results from different plant covers.

2.1.1 Introduction and Objectives of the three experiments

The first, second and third experiments were planned in order to accomplish the first objective of the thesis: to evaluate the transpiration intensity, at different level of substrate moisture content (SMC), of C3 species such as *B. erectus*, *L. corniculatus* and *Salvia officinalis* , of C4 species, such as *C. dactylon*, and of CAM-facultative species such as *S. lydium* and *S. kamtschaticum.* When the substrate water content decreases, these species show different water use strategies, a more or less intense transpiration and, consequently, different cooling performances for the green roof surface, where they grow.

It is well known that CAM-facultative species, even under well-watered conditions (Blanusa *et al*., 2013), have lower transpiration than C3 and C4 species. It is instead not well known how much and how long C3 and C4 species continue to transpire when the moisture content (SMC) of a commercial substrate for green roof progressively decreases and CAM-facultative species switch on the CAM metabolism (no diurnal transpiration), and which behaviour C3 and C4 species show.

In particular, in the first experiment the value of SMC that induces the CAM-facultative species (*S. lydium* and *S. kamtschaticum*) to shift from the C3 metabolism to the CAM metabolism, strongly diminishing the diurnal transpiration in response to water stress, was investigated, as well as the different water losses of CAM-facultative and C3 species when SMC progressively decreases.

The second experiment investigated how much the different gas exchanges of CAM-facultative and C3 plants influenced the substrate temperatures through measurements of their net CO_2 assimilation rates and their leaves and substrate temperatures.

The third experiment is very similar to the first one, but investigated at the same time the three ways of water use (CAM-facultative C3, C4), comparing among them the behaviours of *S. kamtschaticum, B. erectus* and *C. dactylon* when SMC progressively decreases.

2.2 FIRST EXPERIMENT: Daily water loss rates in two CAM-facultative species (*S. lydium* and *S. kamtschaticum*) and two C3 species (*L. corniculatus* and *B. erectus*)

2.2.1 Introduction and Objectives

High temperature and substrate moisture deficit are common on green roofs during the summer season. Crassulacean acid metabolism (CAM) allows succulent species growing on green roofs to survive under drought, due to the day-time stomatal closure and malate production (Dark CO_2 fixation). This reduces the transpiration but, consequently, the cooling effect due to the transpiration itself. In this study we compared the transpiration rates of two CAM-facultative species (*S. lydium* and *S. kamtschaticum*) and two C3 species of semi-xeric grasslands (*L. corniculatus* and *B. erectus*). CAM-facultative species switch between C3 and CAM photosynthesis to respond to environmental conditions, for instance lowering of substrate moisture content (SMC). Our aims were to understand the capacity of selected species to continue the evapotranspiration process, as the substrate moisture content declines and to establish when the CAM behavior occurs in CAM-facultative *Sedum* species.

2.2.2 Materials and Methods

Experiment lasted 36 days and it was carried out in the glasshouse of the DipSA Department (University of Bologna), with 14°/26°C night/day temperature regime that guarantees a good control of the environmental parameters of the glasshouse.

The tested plant species were two CAM-facultative species (*S. lydium* and *S. kamtschaticum*) and two native C3 species (*L. corniculatus* and *B. erectus*). *S. lydium* and *S. kamtschaticum* are of commercial provenance;

L. corniculatus and *B. erectus* were collected from semi-xeric semi-natural grasslands of the Emilian Apennines.

Plants were grown in boxes (40 cm x 30 cm x 22 cm height), where the Harpo/SEIC green roofs stratification (water retention layer, drainage layer and filtering layer) was reproduced (Annexes 2,3). Three replicate boxes for each species were prepared. Each box was filled with the same plant biomass (about 160 g) and the same substrate layer (9 cm thick), equivalent to 10.2 kg of commercial green roof substrate (Harpo/SEIC extensive substrate). In addition, three control boxes, containing just 10.2 kg of bare substrate, were prepared and kept under the same conditions as the vegetated boxes. At the beginning of the experiment, all the 15 boxes were watered till the saturation of the substrate (corresponding, on average, to SMC 26 % weight of water/weight of the substrate and weighted by an electronic balance (MyScale, 40 kg / 2g). The SMC at the start of the experiment was obtained by gravimetric method (Lowery *et al.*,1996): [(wet substrate – dry substrate)/dry substrate]*100. No supplementary water was provided until the SMC reached 3.5 % w/w. SMC decreased over the course of the experiment, that ended for each plant species when the SMC of the substrate reached extremely low values (about 3.5 % w/w).

During the experiment the following parameters were measured:

- the water content of each box, every 12 hours (at 7 am and 7 pm), by gravimetric method, using the electronic balance. The values were expressed as a percentage (w/w) calculated on the total water content at the beginning of the experiment. On Saturday and Sunday no weight measures were performed. These values were used to calculate the SMC during the experiment.
- the diurnal water loss of each box, every 24 hours (at 7 pm), by gravimetric method. The daily water loss was expressed as a percentage (w/w) calculated on the total water content at the beginning of the experiment. It represents the percentage of water loss every day between 7 am and 7 pm. On Saturday and Sunday no weight measures were performed.
- the diurnal evaporation rate, measured every 24 hours as above, of the boxes with the bare substrate only.
- the leaf relative water content (RWC) for all the studied species to monitor plant water status in terms of physiological consequence of cellular water deficit. 5 leaves for each box (15 samples for each species) where collected three times a week following the procedure described by Peñuelas *et al.*, *(*1999).
- the nocturnal malate accumulation in *S. lydium* and *S. kamtschaticum* leaves, three times a week, through the titration method with NaOH following Ting and Hanscom, (1977). 3 leaves/box (9 samples for each species) where collected. Leaf samples were grinded in distilled water, centrifuged at 14000 rpm for 2' and titrated to pH 7 with 0.01N NaOH. Nocturnal malate accumulation of each species at a given date, was expressed as the average value (ml of titrant) of the results of 9 titrations (9 leaves).

2.2.3 Results

The average diurnal water losses ("diurnal" means that they are the percentage of water loss between 7 am and 7 pm every day) during the whole experiment, for the vegetated boxes and the bare substrate are shown in Figure 2.2.1. The average percentages of diurnal water losses, (measured as above) on the whole experiment, for the same boxes, are shown in Table 2.2.1

Table 2.2.1 - Average values and standard deviation, on the whole experiment, of the diurnal water losses (measured at 7 pm every 24 hours as % w/w) for the planted boxes and the bare substrate.

B. erectus	***L. corniculatus***	***S. kamtschaticum***	***S. lydium***	**Bare substrate**
4.0 % ± 0.9 99.4 g/day	4.0 ± 0.5 99.2 g/day	1.6 ± 1.1 45.5 g/day	1.5 ± 0.8 46.5 g/day	1.3 ± 0.7 31.9 g/day
F = 32.67, P = 4.59 E^{-16}				

By Table 2.2.1, the boxes planted with *B. erectus* and *L. corniculatus* shown average diurnal water losses significantly higher than those planted with *Sedum* species and those with the bare substrate only. The vegetated boxes lost, on average, the 4 % of the initial water content. This value is about twice the average diurnal water loss of boxes with *Sedum* species, and about three times the average diurnal water loss of boxes with the bare substrate.

In Figure 2.2.1, it is possible to observe, day by day, the details of the previous results. Boxes with *B. erectus* and *L. corniculatus* lost, on average, the same diurnal amount of water. However, some interesting differences were found. Boxes planted with *B. erectus* lost more water, than boxes planted with *L. corniculatus* till the 5th day, After, starting from the day 8th and, more evidently, from the day 9th, the water losses are higher for boxes planted with *L. corniculatus*, till the end of the experiment at the 12th day. Water losses of the boxes planted with *S. lydium* were higher than those of the boxes with the bare substrate, almost during the whole experiment. Only during the period between 17th day to 26th day, *S. lydium* lost as much water as bare substrate (or slightly less). *S. kamtschaticum* instead, till the 12th day lost more water than *S. lydium* and bare substrate, but from the 16th day and 17th began to lost less water, respectively, than *S. lydium* and bare substrate. This reduction in *Sedum*'s diurnal water losses probably corresponded with the shift from the C3 metabolism to the CAM metabolism.

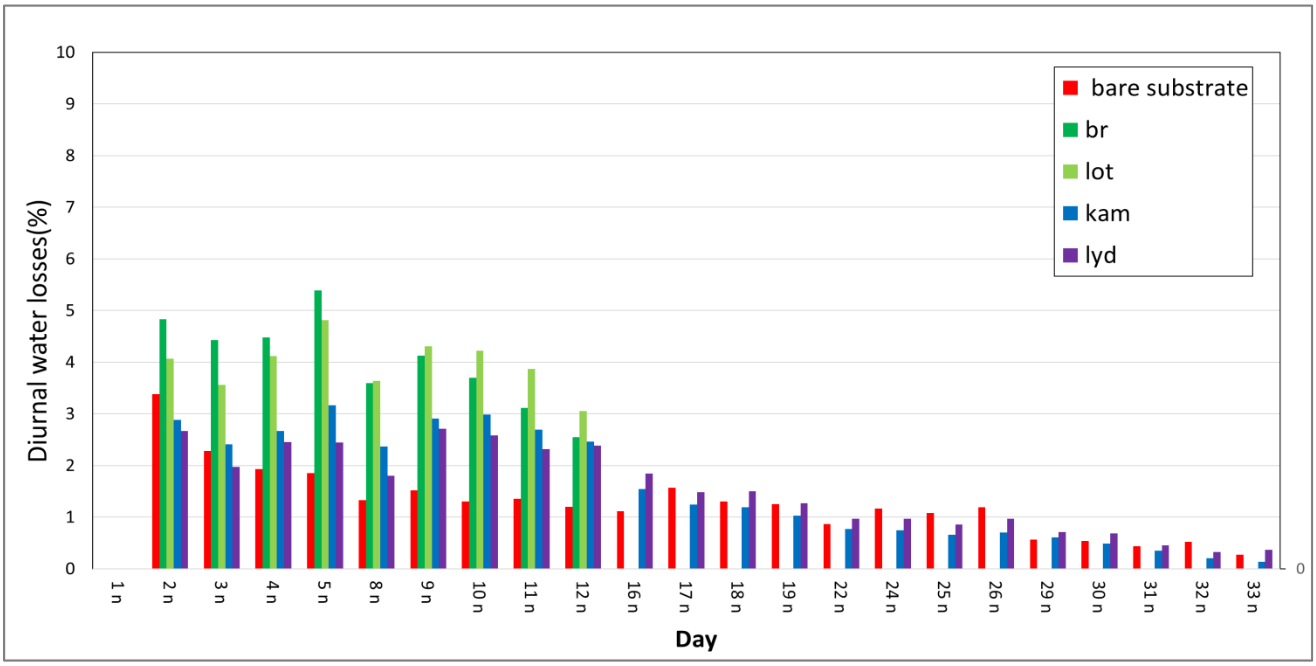

Figure 2.2.1 – Average diurnal water losses (percentage of the SMC calculated on the total water content at the beginning of the experiment) of the vegetated boxes and the bare substrate measured by gravimetric method every 24 hours at 7 pm ("n"). The missing days correspond to weekends or other days when the measurements were not done. br = *B. erectus*, lot = *L. corniculatus*, lyd = *S. lydium*, kam = *S. kamtschaticum*.

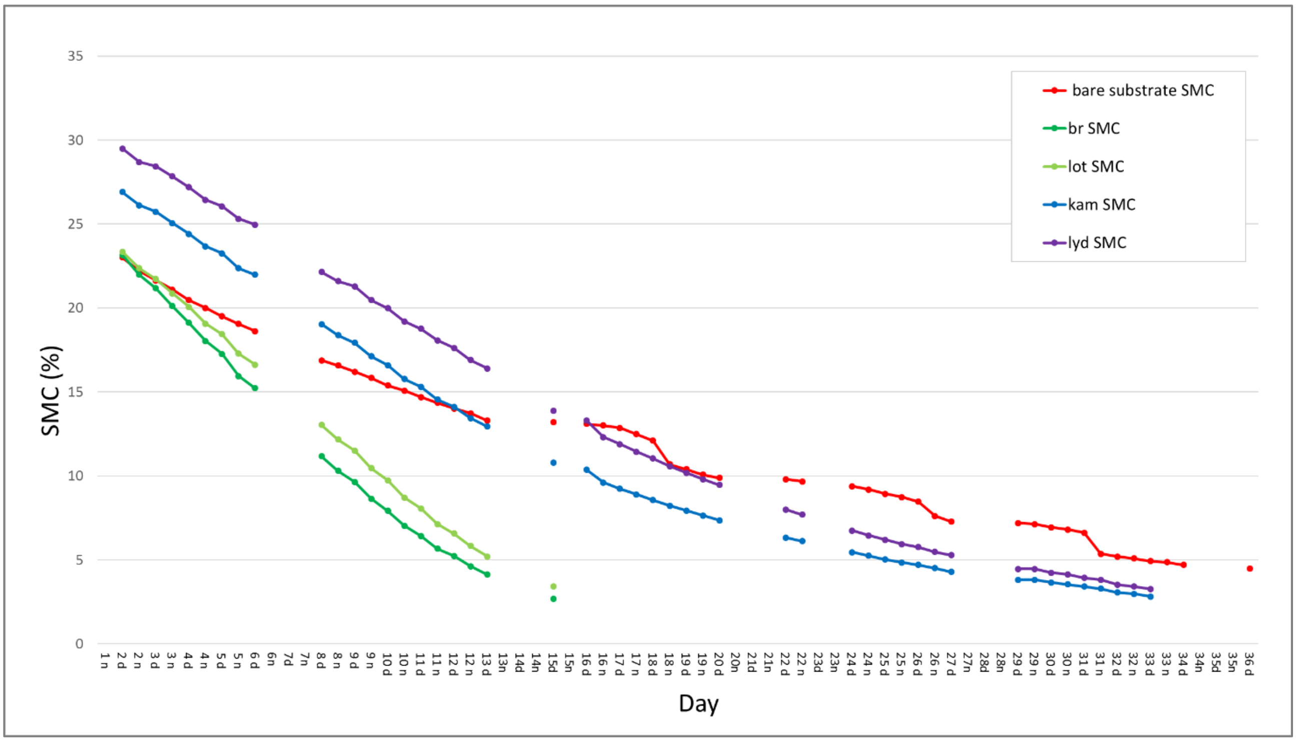

Figure 2.2.2 – Average substrate moisture content (SMC) measured by gravimetric method every 12 hours (n = night, at 7 pm; d = day, at 7 am) for vegetated and non-vegetated (bare substrate) boxes. The missing data correspond to weekends or other days when the measurements were not done. br = *B. erectus*, lot = *L. corniculatus*, lyd = *S. lydium*, kam = *S. kamtschaticum*.

As far as the SMC is concerned, Figure 2.2.2 shows a quick decrease of this parameter for boxes planted with *B. erectus* and *L. corniculatus* (on average, from 23 % w/w to 4 % w/w in 15 days). Boxes planted with *Sedum* species, instead, reached the same SMC value (about 3 % w/w) at the 32th - 33th day of the experiment, Non-vegetated boxes never reached this SMC value; at the end of the experiment the SMC is a little less than 5 % w/w.

During the first 12 days of the experiment, boxes vegetated with the two *Sedum* species maintained higher SMC values that the boxes with bare substrate. During the days between the 13th and the 16th, *S. kamtschaticum* firstly and *S. lydium* secondly, started to increase their diurnal water losses, so the SMC of the boxes vegetated with the two *Sedum* species decreased more quickly than that of the boxes with the bare substrate. This could be due to the physiological control by the two species on the internal water content when the SMC falls below a given threshold value.

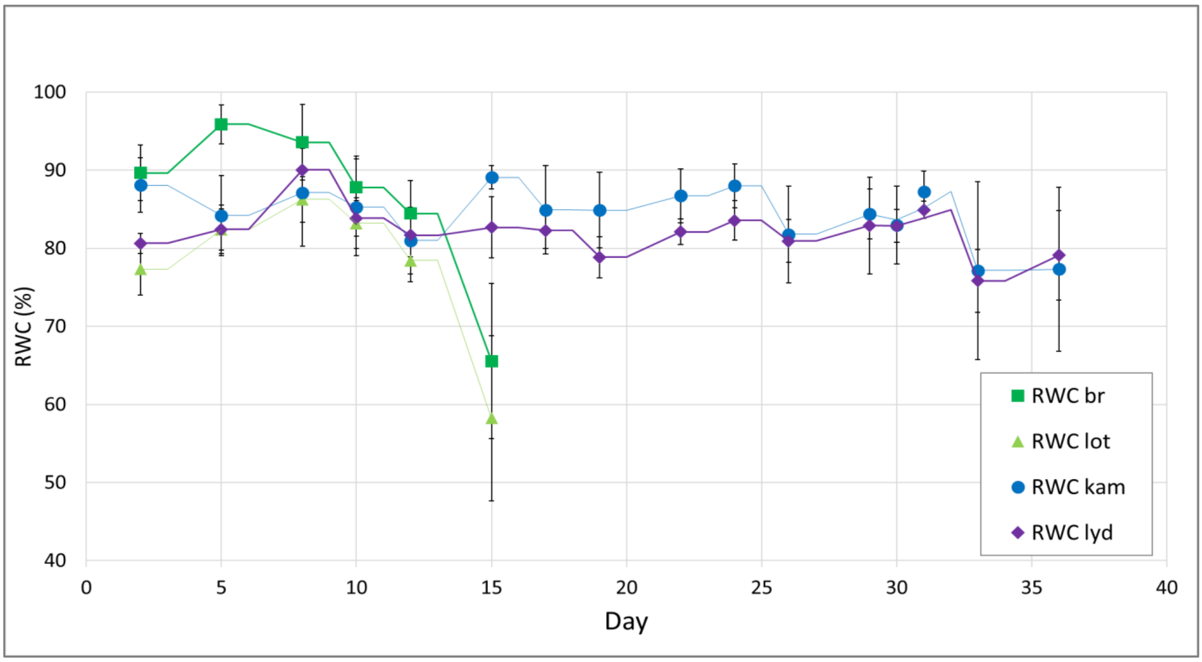

Figure 2.2.3 – Relative Water Content (RWC) ± sd measured three times a week at 12 am. br = *B. erectus*, lot = *L. corniculatus*, lyd = *S. lydium*, kam = *S. kamtschaticum*.

Analyzing the RWC values (Figure 2.2.3), it is possible to highlight the different behavior of *B. erectus* and *L. corniculatus* on one side, and of *S. lydium* and *S. kamtschaticum* on the other. The two C3 species showed a rapid decline of RWC until to extremely low values at the 15th day (58.2 % in *L. corniculatus* and 65.5 % *B. erectus*). The two *Sedum* species maintained values ranging from 80 % to 90 %; only at the end of the experiment the RWC values of *S. lydium* and *S. kamtschaticum* fall down below 80 %. It is interesting to note that at the day 12th *S. lydium* and *S. kamtschaticum* reached RWC values very close to 80 % and, around the same time, the SMC values in the boxes vegetated with the *Sedum* species started to become lower than the SMC value of the boxes with the bare substrate (Figure 2.2.2).

The different behavior of *B. erectus* and *L. corniculatus* on one side, and of *S. lydium* and *S. kamtschaticum* on the other is supported by the values of the RWC shown in Table 2.2.3. The average values of RWC in *S. lydium* and *S. kamtschaticum* are, respectively 82.2 % and 84.4 %; they are not so different from the average value of the RWC of *B. erectus*; however the standard deviation for *B. erectus* is higher than the standard deviation for *S. lydium* and *S. kamtschaticum*. Similar conclusions are reached also comparing the RWC values (and the corresponding values of standard deviation) of the *Sedum* species with those of the second C3 species tested; *L. corniculatus*, which also showed lower RWC values that *B. erectus*, but always high standard deviation values. This demonstrates that the two CAM-facultative *Sedum* species maintained their internal water content more constant than the C3 species.

Table 2.2.2 – Maximum, minimum and average values of RWC (%) calculated during the experiment for each species. Values of sd and results of ANOVA are also reported.

	RWC (%) max	RWC (%) min	Average
B. erectus	95.9	65.5	86.1 ± 10.9
L. corniculatus	86.3	58.2	77.6 ± 10.0
S. kamtschaticum	89.1	77.1	84.4 ± 3.6
S. lydium	90.0	75.8	82.2 ± 3.1
F = 2.54, P = 0.0701495			

The relationships between SMC and RWC (%) (Figure 2.2.4) as well as the correlation coefficient calculated for each studied species (Table 2.2.4), support what has been said above.

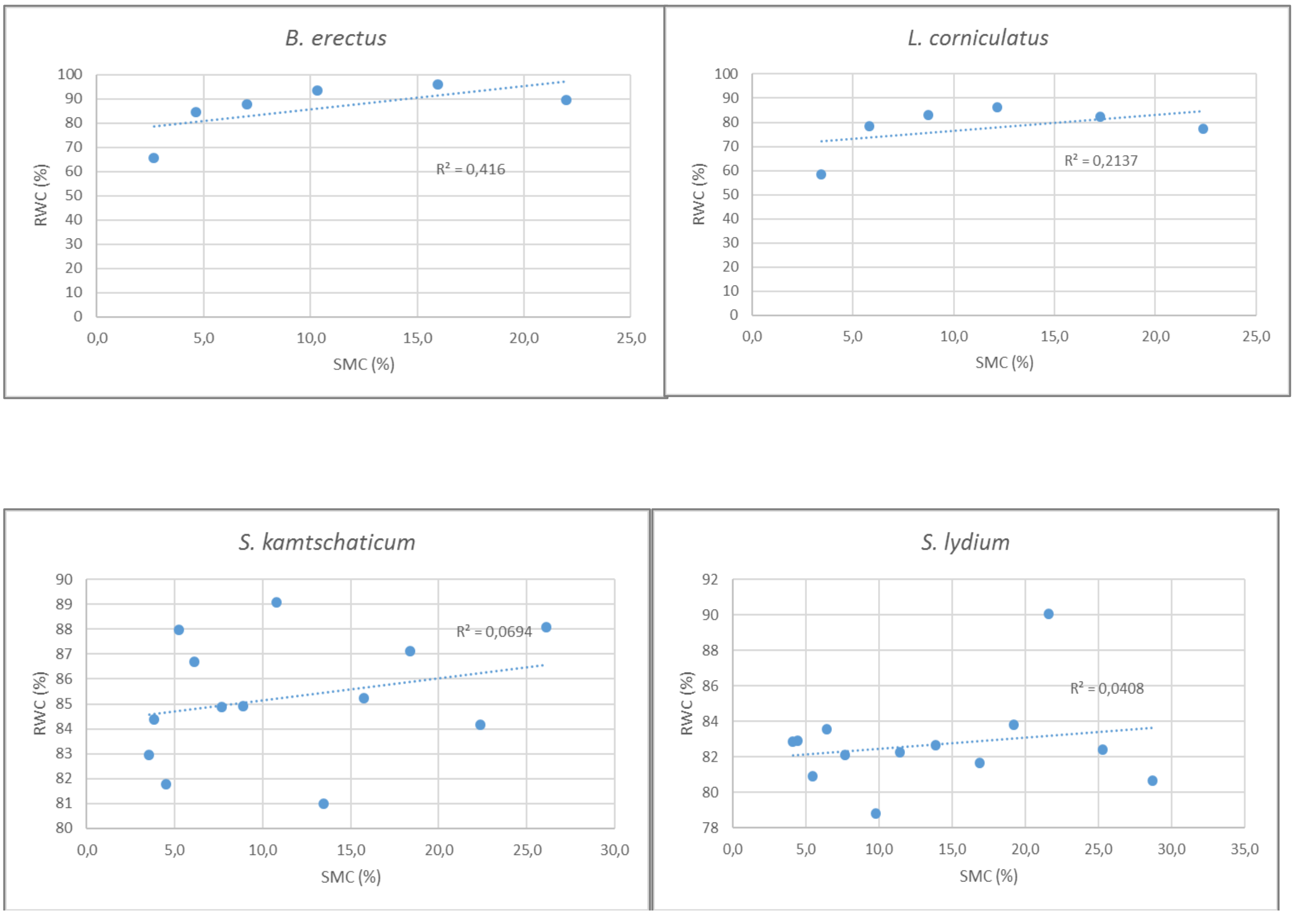

Figure 2.2.4 – Relationships between SMC and RWC (%) for the four studied species.

Table 2.2.3 – Correlation coefficient (R, R^2 and p values) between SMC and RWC (%) for the four studied species.

	R	**R^2**	**P**
B. erectus	0.6450	0.4160	P = 0.080, n = 6
L. corniculatus	0.4623	0.2137	P = 0.294, n = 6
S. kamtschaticum	0.2634	0.0694	P = 0.423, n = 13
S. lydium	0.2020	0.0408	P = 0.603, n = 13

B. erectus shown the best value of the correlation coefficient between the two parameters. Its RWC values depend (P = 0.080) from the SMC. The RWC values of *S. lydium* and *S. kamtschaticum* are instead weakly dependent (P = 0.603 for *S. lydium*; P = 0.423 for *S. kamtschaticum*) from the SMC of the boxes where they growth. *L. corniculatus* shown an intermediate behaviour.

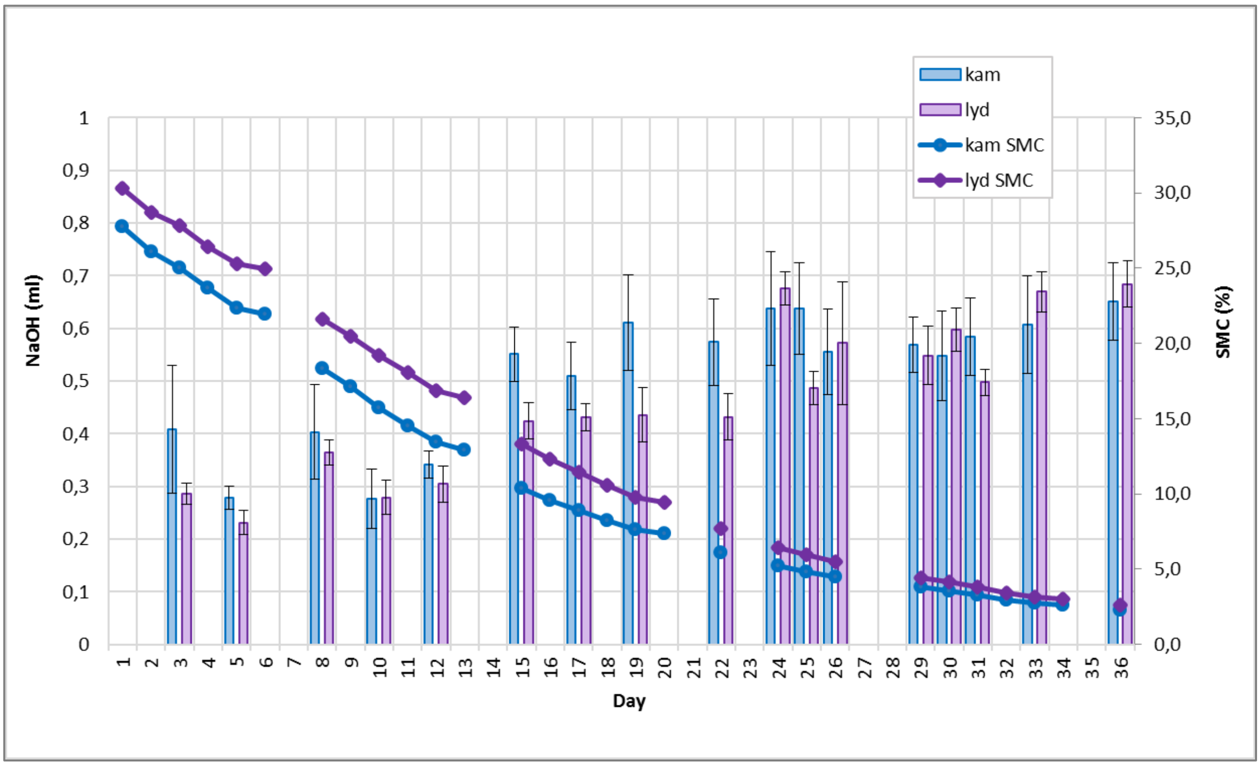

Figure 2.2.5 – Averages values ± sd of malate concentration in leaves of *S. lydium* and *S. kamtschaticum* during the experiment (bars). Malate concentration is evaluated as ml of NaOH necessary for the titration. The average values (lines) of SMC in the boxes planted with the same species are also shown. lyd = *S. lydium,* kam = *S. kamtschaticum*.

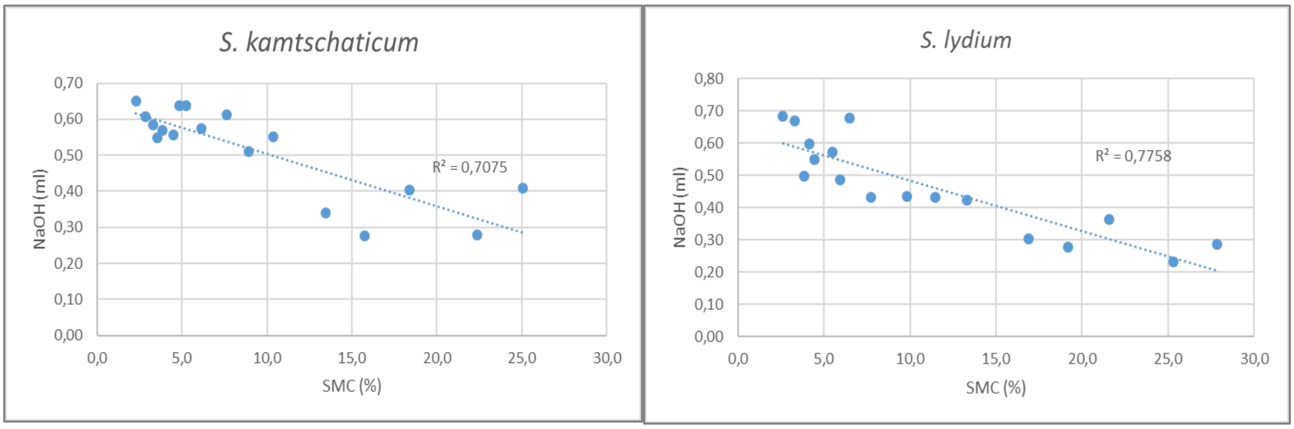

Figure 2.2.6 – Relationships between the SMC percentages and the malate concentration in leaves (amount of NaOH necessary for the titration) of S. *kamtschaticum* and *S. lydium*.

This experiment aimed also to determine which are the values of SMC that switch-on the CAM metabolism in the two CAM-facultative species of *Sedum.* An increase of nocturnal malate concentration in the vacuole is a good indicator of the activation of the CAM metabolism. During the night, in fact, a plant employing CAM metabolism has its stomata open, allowing CO_2 to enter and be fixed as organic acids stored in the vacuoles. In this experiment, malate concentration was evaluated through the titration method with NaOH following Ting and Hanscom, (1977).

Results of the titration analyses performed on leaves of *S. lydium* and S. *kamtschaticum* are shown in Figure 2.2.5. In the same figure, the average values of SMC in the boxes planted with the two *Sedum* species are shown. Figure 2.2.6 shows the relationships between these two parameters. They were significantly negatively correlated (R = 0.8411, R^2 = 0.7075, $P < 0.001$, n = 17 for *S. kamtschaticum*; R =0.8808, R^2 = 0.7758, $P < 0.001$, n = 17 for *S. lydium*). However, the behavior of the two species was different. The nocturnal malate accumulation in *S. kamtschaticum* sharply increased at day 15th from the start of the experiment, when the SMC was about 10 % w/w. At the same date the SMC of boxes with *S. lydium* was higher, about 13 % w/w, and malate accumulation showed an inconspicuous increase. It is interesting to note that in *S. lydium* the malate accumulation was basically less abundant than in *S. kamtschaticum*, even at equal values of SMC. At day 22 from the start of the experiment, for example, when the SMC was on average equal to 7.7 % w/w in the boxes with *S. lydium*, the titration values with NaOH for the malate accumulation was on average equal to 0.43 ml. At day 19th, instead, when the SMC was on average equal to 7.6 % w/w for *S. kamtschaticum*, as above at day 22 for *S. lydium*, the titration values with NaOH for the malate accumulation was on average equal to 0.61 ml.

From the 24th day, in *S. lydium*, the titration values with NaOH for the nocturnal malate accumulation showed a second increment (from 0.43 ml to 0.68 ml).

The behavior of the two species was not so different only from day 24 onwards, when the SMC of the boxes with *S. lydiun* and *S. kamtschaticum* became very low (from about 6 % to 2.5 % w/w).

Assuming that the first recorded increase of malate concentration for *S. kamtschaticum* (day 15) corresponded to the shift of the species metabolism from C3 to CAM, this shift took place when the RWC values were still high (about 89 %). Not so different were the values of *S. kamtschaticum* RWC (about 88 %), when the second high value of malate concentration was recorded (day 24).

Regarding to *S. lydium,* the two increments of malate observed at 15th and 24th day, made the identification of the shift of the species metabolism more complicated than for *S. kamtschaticum*.

However, even for *S. lydium*, the values of RWC recorded at 15th and 24th day, are particularly high (about 83 %).

In Table 2.2.3 the daily water losses of the planted boxes and of the bare substrate boxes are compared, at different values of SMC. At about the 11 % w/w of SMC, when *S. kamtschaticum* shifts to the CAM metabolism and the boxes where it grows significantly decreases their diurnal water losses (day 16 in Figure 2.2.2), C3 species such as *B. erectus* and *L. corniculatus* continued to lose more water than *Sedum* ones (approximately two times more).

Under 6 % w/w of SMC, the gap between C3 species and CAM-facultative *Sedum* increased. *B. erectus* and *L. corniculatus*, respectively, lost three and four time more water (% w/w) than *S. kamtschaticum* and *S. lydium.*

Table 2.2.3 – Mean diurnal water loss (± sd) of the studied species and of the bare substrate at different SMC values. For each value of SMC, results of the ANOVA on the mean daily water loss (%) are also reported.

SMC about 20 % w/w (Well-watered condition)		
	Mean diurnal water loss (%)	**Mean diurnal water loss (g)**
bare substrate	1.9 ± 0.1	46.6 g
B. erectus	4.4 ± 0.1	109.3 g
L. corniculatus	3.6 ± 0.3	89.2 g
S. kamtschaticum	2.4 ± 0.2	68.3 g
S. lydium	1.8 ± 0.1	55.8 g
F = 104.20, P = 4.15 E^{-08} LSD = 0.4, P = 0.05		
SMC about 11 % w/w (First malate increase for *S. kamtschaticum*)		
	Mean diurnal water loss (%)	**Mean diurnal water loss (g)**
bare substrate	1.2 ± 0.1	29.5 g
B. erectus	3.6 ± 0.3	89.5 g
L. corniculatus	3.6 ± 0.3	89.2 g
S. kamtschaticum	1.5 ± 0.1	42.7 g
S. lydium	1.5 ± 0.1	46.5 g
F = 75.25, P = 2.01 E-07 LSD = 0.7, P = 0.05		
SMC about 6 % w/w (Second malate increase for *S. lydium*)		
	Mean diurnal water loss (%)	**Mean diurnal water loss (g)**
bare substrate	0.4 ± 0.1	9.8 g
B. erectus	3.1 ± 0.1	77.0 g
L. corniculatus	3.1 ± 0.3	77.8 g
S. kamtschaticum	0.3 ± 0.1	8.5 g
S. lydium	0.9 ± 0.1	27.9 g
F = 213.28, P = 1.24 E^{-09} LSD = 0.3, P = 0.05		

2.2.4 Discussion and Conclusions - first experiment

The first experiment has analysed in depth the transpiration of *B. erectus*, *L. corniculatus*, *S. lydium* and *S. kamtschaticum* when the available water decreases, under 14°/26°C night/day temperature regime and other experimental conditions as described in Materials and Methods.

Results obtained confirmed the different behavior of C3 species such as *B. erectus* and *L. corniculatus*, and of CAM-facultative species such as *S. lydium* and *S. kamtschaticum.*

B. erectus and *L. corniculatus* continued to transpire even at very low value of SMC (around 6 % w/w), *S. lydium* and *S. kamtschaticum*, which since the first days of the experiment showed the lower diurnal water losses, reduced further their diurnal transpiration very early, even at considerable values of SMC.

This further reduction of diurnal water losses of *Sedum* species coincides with a significant increase of the malate accumulated during the night (from 11 % to 6 % w/w of SMC) therefore, the reduction in transpiration is due to the activation of CAM metabolism in optional CAM species.

Comparing diurnal water loss rates of *B. erectus* and *L. corniculatus* with those of the bare substrate (Table 2.2.3), the two C3 species, in theory, can efficaciously contribute in cooling performances of a green roof.

Comparing diurnal water loss rates of *Sedum* species with those of the bare substrate (Table 2.2.3), the scarce potential of CAM-facultative plants in cooling performances of a green roof evidently emerges (Vaz Monteiro *et al*., 2017).

On the other hand, a consequence of this different transpiration behavior is a faster decline of SMC and RWC in *B. erectus* and *L. corniculatus* than in *Sedum* species. If the better cooling performances of *B. erectus* and *L. corniculatus* are required, irrigation is necessary/mandatory. On the basis of the relationships highlighted between SMC and transpiration intensity, it could be possible to choose what is the value of SMC that allows to maintain a good cooling effect with a moderate supply of irrigation water.

2.3 SECOND EXPERIMENT: The influence of the gas exchange and transpiration of C3 and CAM-facultative species on the green roof's cooling performance

2.3.1 Paper presentation

The microclimatic cooling effect of urban green spaces is well documented (Wolch *et al.*, 2014), in a green roof context, microclimatic cooling refers to the process whereby the air over the roof is cooled by evapotranspiration and shading effect (Shashua-Bar *et al.*, 2009) due to both the roof components and the plants (Gaffin *et al*., 2006; Wolf and Lundholm, 2008; Blanusa *et al*., 2013). Moreover, substrate and membrane layers show significant insulation properties well documented by many studies (Del Barrio, 1998; Eumorfopoulou and Aravantinos, 1998; Niachou *et al.*, 2001; Onmura *et al*., 2001; Theodosiou, 2003; Liu, 2004; Liu and Minor, 2005). Shading and reflection by vegetation can also contribute to cooling (e.g. Vaz Monteiro., 2017). At the same time, other experimental studies suggest that the a significant component of green roofs cooling is derived from evapotranspiration (Gaffin *et al*., 2005; Gaffin *et al*., 2006; Wolf and Lundholm, 2008), with up to 30 % of total cooling due to transpiration from plants, through the absorption of the heat from the air in the process of latent heat flux (Takakura *et al*., 2000). Transpiration rate of plants is thus determinant of the thermal functioning of green roof ecosystems. Consequently, it is obvious that the choice of plant species, according to the transpiration capacity, to use on green roof have a significant impact on the green roof cooling capacity during the summer season. CAM metabolism allows succulent species growing on green roofs to survive under drought, due to the day-time stomatal closure and malate production. This reduces the transpiration but, consequently also the cooling effect due to the transpiration itself. On the other hand, the C_3 plant species show higher transpiration rate, but this makes them more vulnerable and more susceptible to death during long periods of drought. For these reasons, it is worth investigating the use of C_3 native species from xeric and semi-xeric habitats. Native plants have evolved to grow and survive in their regional microclimatic conditions, pests and diseases (White and Snodgrass, 2003; Dewey *et al*., 2004) and in the Mediterranean area this includes extreme summer weather (without resorting to CAM metabolism).

The second experiment was described in the paper accepted for the publication in special issue of Acta Horticulturae IRHS, "Greener Cities 2017". It was performed at the School of Agriculture, Policy and Development, University of Reading (UK), during a four months stage under the tutorship of Dr. Tijana Blanusa (Principal Horticultural Scientist – Royal Horticural Society) and prof. Paul Hadley (Professor). During this abroad period, we analyzed the net CO_2 assimilation rates (A) activities of two CAM-facultative species (*Sedum lydium* Boiss and *Sedum kamtschaticum* Fisch.) and three C3 species (*Bromus erectus* Huds., *Lotus corniculatus* L., *Salvia officinalis* L.), during a gradual reduction of moisture content in the growing medium layer. This experiment aimed to confirm (and improve) the results of the first experiment. Through measures of net CO_2 assimilation rate, the transpiration activity of C3 species under drought conditions was measured. In addition, measuring the surface temperatures of the experimental boxes through thermocouples, it was possible to observe directly if C3 species could have better cooling capacity than CAM-facultative species, under well-watered and drought stress conditions.

Initially, leaf stomatal conductance (g_s) measurement was planned using the porometer (AP4, Delta-T device, Cambridge, Cambridgeshire, UK), but preliminary results showed the incompatibility between *S. lydium* and *S. officinalis* leaves and the porometer because of the problems due to the soft and small leaves of *S. lydium* and the hairy leaves of *S. officinalis*, in particular under draught condition. Because of many problems between porometer and *S. officinalis* and *S. lydium*, it was necessary to use only the net CO_2 assimilation rates parameter to obtain an estimate of the plant's transpiration activity of all species.

2.3.2 Preliminary concepts

2.3.2.1 Leaf gas exchange measurements (A)

Leaf gas exchange measurements provide direct measure of the net rate of photosynthetic CO_2 assimilation. Conventional gas exchange techniques measure fluxes of water and CO_2 into and out of a leaves. The gradient in partial pressure of CO_2 from ambient air (p_a) to the substomatal cavities (p_i) is derived using Ficks law of diffusion, which states that the gradient in partial pressure is equal to the flux divided by the conductance, i.e. $p_a - p_i = A/g_s$, where A is the rate of CO_2 assimilation (or Net Assimilation rate) and g_s is the stomatal conductance to CO_2 (Evans and Caemmerer, 1996).

Main advantages of gas exchange measurements: instantaneous, non-destructive and direct. CO_2 exchange systems use enclosure methods, where the leaves are closed in a transparent chamber.

2.3.2.2 Leaf Area Index (LAI)

The leaf area index (LAI) is the main variable used to model canopy photosynthesis and evapotranspiration (Weiss *et al*., 2004). It defines the size of the plant–atmosphere interface and thus plays a key role in the exchange of energy and mass between the canopy and the atmosphere (Weiss *et al*., 2004). Values of LAI depend on plant type, and are typically in the range of 0.5–5.0 (Berardi *et al*., 2014). It was measured at the end of the second experiment by dividing the leaf area of *S. officinalis, B. erectus, L. corniculatus, S. lydium* and *S. kamtschaticum* (measured with Area Meter, Delta-T Devices Ltd., Cambridge, UK) by the surface area from which the leaves were sampled (six samples per box) as suggested by Blanusa *et al*. (2013).

2.3.3 The influence of the gas exchange and transpiration of C_3 and CAM-facultative species on the green roof's cooling performance

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Abstract

Crassulacean acid metabolism (CAM) allows succulent species to survive under drought by reducing the daytime water loss and gas exchange. We compared gas exchange of two CAM-facultative species (*Sedum lydium* Boiss. and *Sedum kamtschaticum* Fisch.) and three C_3 species of semi-xeric habitats (*Lotus corniculatus* L., *Bromus erectus* Huds. and *Salvia officinalis* L.) during a gradual reduction of substrate moisture content (SMC). The aim was to describe how the C_3 and the CAM-facultative species modify their gas exchange rates when the substrate dries out gradually, as well as to identify the critical value of SMC that stops the transpiration. Additionally, we tested the cooling capacity of specie in a week-long glasshouse experiment, in vegetated containers with commercial green roof substrate. The net CO_2 assimilation (A) rates of C_3 species were significantly higher than those of CAM-facultative species. When SMC fell below 12 % v/v, *Sedum* species showed a rapid decline of A due to the likely switch from C_3 to CAM metabolism. However, the high rates of gas exchange of C_3 species caused a rapid decline of RWC over the course of the experiment and death of some leaves. The lower substrate temperatures under C_3 plants confirmed the positive effect of high gass exchange rates and transpiration on the cooling capacity. We conclude that the use of C_3 plants, with sustainable irrigation (to maintain at least, on average, 10 % v/v of SMC), has a potential to provide greater cooling to a green roof than the use of *Sedum* species.

Keywords: Green Infrastructures (GI), cooling effect, wild plants, Net Assimilation rate (A).

Introduction

Sedum species (family *Crassulaceae*) are a popular plant choice on green roofs thanks to their ability to survive drought by activating the facultative Crassulacean Acid Metabolism (CAM) (Herrera, 2009). Facultative CAM plants reduce daytime gas exchange and transpiration under drought conditions, which aids survival but decreases the transpirational cooling performance of green roofs. Many C_3 wild plant species in the Mediterranean area, however, are adapted to grow under harsh conditions without the use of CAM metabolism and could thus represent an important resource for increasing the pool of species for green roofs (Caneva et al., 2013: Vestrella *et al*., 2015). In this paper we compared the Net CO_2 Assimilation (A) rates of two *Sedum* facultative CAM species (*Sedum lydium* Boiss and *Sedum kamtschaticum* Fisch.) and three C_3 species (*Bromus erectus* Huds., *Lotus corniculatus* L., *Salvia officinalis* L.) during a gradual reduction of moisture content in the growing medium. The aim was to observe if the evapo-transpiration (estimated via net assimilation rate) of C_3 species is higher than *Sedum*. Also to establish a substrate moisture level at which *Sedum* species change their metabolism from C_3 to CAM and, consequently, deduce if C_3 species could have better cooling capacity than facultative CAM species under both well-watered and drought conditions.

Materials and Methods

This study was conducted in the ventilated glasshouses within the School of Agriculture, Policy and Development, University of Reading (UK). Twelve boxes ((l) 43.5 cm x (w) 35.0 cm x (d) 9.0 cm) were prepared, two for each plant species tested and two with bare substrate (control). All boxes were filled with the same weight of green roof substrate (Shire Extensive Substrate, Shire Minerals, UK) and they were planted to achieve full ground coverage (Annex 4). All boxes were well watered until the field capacity (on average 29 % v/v**)** 24 h before the start and then, left to dry until reaching critical values of substrate moisture content (under 5 % v/v). During the experimental period (from 14 June to 21 June), the mean daytime air temperature ranged 23 °C – 30 °C.

Net CO_2 assimilation rate (A) was measured daily, between 12 pm and 4 pm, using portable infrared gas analyzer (LCi Portable Photosynthesis System, ADC BioScientific Ltd, Hoddesdon, UK) , on five leaves or sprigs per box. Except for *S. officinalis*, it was impossible to fit individual leaves into the analyser’s cuvette, so sprigs containing several leaves (and covering the entire cuvette) were chosen and repeatedly measured. At the end of the experiment, the leaf areas of each spring were determined using a leaf area meter (WinDias 3, Leaf Image Analysis System, Delta-T devices Ltd, Cambridge, UK) and assimilation outputs adjusted where necessary, to relate to the area of the cuvette (6.25 cm^2). The substrate moisture content (SMC) of each box was monitored daily using SM200 probe (Delta-T devices Ltd., Cambridge, UK). The substrate temperatures were monitored continuously using two thermistors (type T fine PTFE insulated twin twisted wires. Accuracy: ± 0.1 °C, resolution: ± 0.1 °C) positioned at 1-2 cm depth in the middle of each box and connected to the DL2e data logger (Delta-T Devices Ltd, Cambridge, UK; Annex 4); data were collected every 1 min and averaged over 30 min. The leaf temperatures were measured daily (concurrently with A measurements) for each box using Infrared Thermal Camera NEC TH7700 (NEC San-ei Instruments Ltd., Japan). Leaf area index (LAI) of each species was determined at the end of the experiment by dividing the plants’ leaf area with the 20 x 20 cm area of the substrate, from which plants were collected. The relative water content (RWC) was determined according to Živčák (2008) at the end of experiment. Data were statistically analysed using GenStat (15th Edition, Lawes Agricultural Trust, Rothamsted Experimental Station, UK) to compare the species for their A and final RWC.

Results

Net assimilation rate

The C_3 species showed a higher assimilation rates than the two *Sedum* species; all species showed a rapid decline of net A (Table 1) from Day 5, as SMC ranged between 13 % v/v (*S. officinalis*) and 9 % v/v (*B. erectus*). The net A rate decreased faster for *B. erectus* and *L. corniculatus* than *Sedum* species and *S. officinalis,* particularly between Days 5 and 6.

Leaf temperatures

All studied species had leaf temperatures that were 1.6-8.8 ^{o}C lower than surface temperatures of bare substrate (Figure 1 A). Between the species, except on Day 3 and on Day 5, *B. erectus, L. corniculatus* and *S. officinalis* showed lower leaf temperatures than *Sedum* species for the whole duration of the experiment. On average, the leaf temperatures of C_3 species were 2.06 ^{o}C lower than the leaf temperatures of two *Sedum* species. Also during the decline of net A (from Day 5) due to a decrease of SMC, all C_3 species continued to show the coolest leaf temperatures, in particular *S. officinalis.*

Table 1. - Mean net assimilation rate (µmol m^{-2} s^{-1}) and the corresponding substrate moisture content (SMC) value for all studies species over the course of the experiment. Net assimilation data are mean of 10 measurements per species. The percentages of SMC (v/v) are mean of six measurement per treatment.

Net assimilation rate and SMC (v/v)	**Day 1 14/6**	**Day 2 15/6**	**Day 3 16/6**	**Day 4 17/6**	**Day 5 18/6**	**Day 6 19/6**	**Day 7 20/6**	**Day 8 21/6**
B. erectus	13.79	12.87	12.83	13.97	12.17	3.85	1.00	-
SMC	29 %	20 %	17 %	14 %	9 %	4 %	2 %	
L. corniculatus	22.76	19.68	22.53	24.14	23.62	11.40	9.32	-
SMC	26 %	20 %	16 %	15 %	11 %	5 %	4 %	
S. officinalis	17.90	15.99	17.97	18.07	17.63	16.36	10.31	4.75
SMC	25 %	21 %	18 %	15 %	13 %	10 %	3 %	2 %
S. lydium	7.71	8.52	9.11	10.41	7.60	5.50	2.60	0.38
SMC	26 %	19 %	17 %	16 %	12 %	10 %	5 %	3 %
S. kamtschaticum	7.13	5.80	6.69	7.14	5.29	2,73	0.78	0.21
SMC	28 %	21 %	19 %	15 %	12 %	6 %	4 %	2 %
Net assimilation least significant difference (LSD)	2.88	3.37	3.60	3.37	2.31	2.73	2.63	1.78

Substrate temperatures

As we expected, substrate temperatures (Figure 1 B) were always cooler than leaf or surface temperatures, but we observed also that the substrate temperatures in all planted boxes with plants were lower than in the boxes without plants. In general, *L. corniculatus* had hottest substrate temperatures among the C_3 species and *S. lydium* showed the hottest temperatures among all plant species considered. In particular, until the Day 4 (under well-watered conditions), *S. officinalis*, *B. erectus* and *S. kamtschaticum* showed the lowest substrate temperatures (by 3.6 °C lower than bare substrate on Day 3 for example). From the Day 5, when SMC decreased under 13 % v/v, all boxes increased their substrate temperatures, but the C_3 species continued to show the coolest substrate temperatures or, in the worst cases, the same substrate temperatures of *Sedum* species (except for *L. corniculatus* on Day 7). On Day 6, for example, the C_3 species and *Sedum* species showed, on average, temperatures 2.8 °C and 1.6 °C respectively lower than bare substrate. On the other hands, *Sedum* species and *S. officinalis*, provided a few cooling effect for one more day than *L. corniculatus* and *B. erectus* for which the experiment ended one day earlier because of their precarious conditions due to water stress.

Final Relative Water Content (RWC)

The C_3 species, especially *L. corniculatus* and *B. erectus*, showed a poor ability to maintain acceptable values of RWC until the end of the experiment, these two species reached critical values of RWC (40 % and 46 % respectively, data not shown) by Day 7. In fact, the leaves of some individuals of *L. corniculatus* and *B. erectus* began to rolling already from Day 6. *S. officinalis* showed, at the end of the experiment (Day 8), the highest RWC values (72 %) among the C_3 species tested. However, also for this species, at the end of the experiment, a few leaves per plant were slightly wilted.

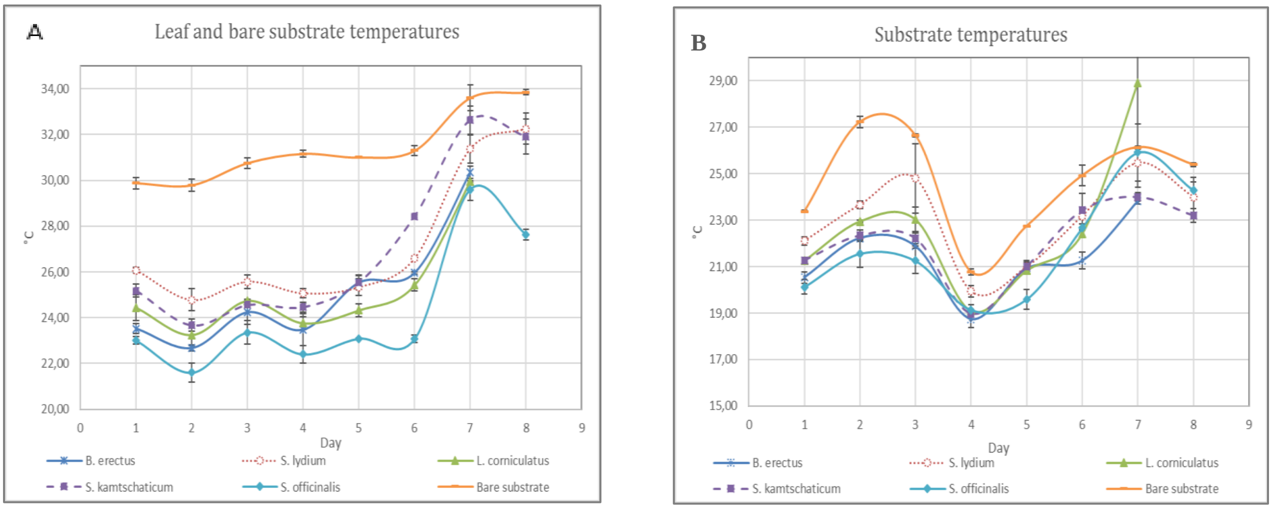

Figure 2 - A) Leaf temperatures and bare substrate temperatures measured between 12 pm and 4 pm. Data are mean of 6 measurements per species and substrate B) Mean substrate temperatures at 1 cm depth for all species and bare substrate. Data are mean of two measurements per treatment.

Discussion

Net assimilation rate, substrate moisture content and final RWC

C_3 species had higher net A (and thus likely, transpiration rates) than *Sedum*. In some cases, net assimilation values of *L. corniculatus* exceeded 3-fold those of *Sedum* (*e.g.* on Day 5 when SMC was already below 12 % v/v). There was a decline of the net assimilation when the substrate moisture reached 12 % v/v in *Sedum* and from 11, 10 and 9 % v/v in *L. corniculatus*, *S. officinalis* and *B. erectus*, respectively. CAM species are known to reduce the net assimilation and transpiration in the periods of water stress (Olivares *et al*., 1984: Borland and Griffiths, 1990). In addition, the results of our previous study (D'Arco *et al*., 2016) showed the malate accumulation during the dark period in the *S. lydium* and *S. kamtschaticum* leaves, at around 13 - 10 % v/v of SMC. It is thus likely that the shift we are seeing in *Sedum* was due to the change from C_3 to CAM metabolism. The final RWC values showed that the A decline in C_3 species is followed by a rapid and significant drop in RWC. *Sedum* species demonstrated to have the capacity to maintain high quantity of water in the leaves also under severe drought (> 87 % of RWC when the SMC values dropped below 6 % v/v). *B. erectus* and *L. corniculatus* plants, which transpired more than *Sedum* species, reached at the end of experiment, values of RWC considered critical (Babu *et al*., 1999) and started to show the visible signs of water stress (rolling leaves and drying). *S. officinalis* is an exception; it transpired more than *Sedum*, but at the end of the experiment, the average RWC values were still higher than the critical limit and no part of plant material was lost.

We suggest that the use of C_3 species (in particular *S. officinalis*) could increase significantly the cooling effect of the green roof through the transpiration at least until about 9 % v/v of SMC.

Leaf and substrate temperatures

Active gas exchange (CO_2 and water vapour) has an important role in the cooling effect; *B. erectus* and *S. officinalis* showed the lowest substrate and leaf temperatures and the highest A values until Day 6 when the SMC dropped to 4 % v/v and 10 % v/v for these species, respectively. Other factors, including light leaf colour and presence of leaf hairs may also explain *Salvia*'s advantage (Vaz Monteiro *et al*., 2016). Despite high A, *L. corniculatus* showed, almost for the whole duration of the experiment, higher substrate temperatures than with *S. kamtschaticum*. This may be explained by the lower LAI of *L. corniculatus* compared to *S. kamtchaticum* (almost four fold). *S. kamtschaticum* could thus provide a positive contribution to the cooling effect by the shading effect of their leaves. Similarly, despite of lower gas exchange of *B. erectus* than *L. corniculatus,* the substrate of *B. erectus* was cooler and the LAI value was higher than *L. corniculatus.* The contribution of *B. erectus* to the cooling effect was likely the sum of a good transpiration activity, but also of shading. Otherwise, *S. officinalis* showed a lower LAI value (4.45) than *B. erectus* and *S. kamtschaticum*, but the substrate temperatures of boxes with *S. officinalis* were the coolest thanks to its high gas exchange activity.

Conclusions

This study demonstrates that all C_3 species tested showed greater gas exchange activity than *S. lydium* and *S. kamtschaticum* under well-watered conditions but, also, under drought (from about 10 % v/v of SMC), when *Sedum* facultative CAM species begin to change their metabolism from C_3 to CAM. The higher gas exchange capacity of C_3 species reduces leaf temperatures and consequently increases the cooling effect of green roofs particularly during the hot and dry weather. However, this study also revealed that high LAI and ground coverage are required in addition to high transpiration activity, to maximize the green roof cooling effect.

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2.4 THIRD EXPERIMENT: Daily water loss rates and decline of SMC in *S. kamtschaticum* (CAM-facultative species), *B. erectus* (C3 species) and *C. dactylon* (C4 species)

2.4.1 Introduction and Objectives

The main aim of this experiment was to compare the diurnal water losses of CAM-facultative, C3 and C4 species and to define how the RWC varies during the decline of substrate moisture content. The species used in this experiment were *Sedum kamtschaticum* Fisch. (CAM-facultative species), *Bromus erectus* Huds. (C3 species) and *Cynodon dactylon* L. (C4 species). As far as these aspects are concerned, the behavior of *S. kamtschaticum* and *B. erectus* were already tested in the first experiment under 14°/26°C night/day temperature regime. The behavior of *C. dactylon* is instead tested for the first time in this experiment.

C. dactylon (Bermuda or Couch grass) is a perennial grass, well known for its adaptation to a variety of habitat types, its distribution is very wide, but it occurs predominantly in tropical and warm temperate regions throughout the world (Hameed and Ashraf, 2008).

2.4.2 Materials and Methods

The experiment was organized in a similar way as the first experiment and carried out in the greenhouse of the Department of Agricultural Science under controlled environmental conditions. The thermal regime, however, was different than that of the first experiment, with night/day average temperature regime of 24°/30°C. *B. erectus* and *C. dactylon* plants were collected from semi-natural habitats around Bologna; *S. kamtschaticum* had a commercial origin. For each species three boxes (40 cm x 30 cm x 22 cm height) were prepared as in the first experiment (Annexes 2,3)t. Each box was filled with the same plant biomass (about 270 g) and same substrate layer (9 cm thick), 10.2 kg of commercial dry green roof substrate (Harpo/SEIC extensive substrate). In addition, three control boxes, containing just dry bare substrate were prepared and kept under the same environmental conditions as the vegetated boxes. They were monitored as control of the diurnal water loss due to evaporation only. At the beginning of the experiment, all boxes were watered to the saturation, on average, 28 % w/w of SMC and no supplementary water was provided during the experiment. The experiment lasted 16 days.

During the experiment the following parameters were measured:

- the water content of each box, every 12 hours (at 7 am and 7 pm), by gravimetric method, using the electronic balance. The values were expressed as a percentage (w/w) calculated on the total water content at the beginning of the experiment. On Saturday and Sunday no weight measures were performed. These values were used to calculate the SMC during the experiment.
- the diurnal water loss of each box, every 24 hours (at 7 pm), by gravimetric method. The daily water loss was expressed as a percentage (w/w) calculated on the total water content at the beginning of the experiment. It represents the percentage of water loss every day between 7 am and 7 pm. On Saturday and Sunday no weight measures were performed.
- the diurnal evaporation rate, measured every 24 hours as above, of the boxes with the bare substrate only.
- the leaf relative water content (RWC) for all the studied species to monitor plant water status in terms of physiological consequence of cellular water deficit. Six times during the experiment (5 leaves x 3 boxes = 15 leaves) following the procedure described by Peñuelas *et al.*, (1999).
- the nocturnal malate accumulation in *S. lydium* and *S. kamtschaticum* leaves, seven times during the experiment, through the titration method with NaOH following Ting and Hanscom, (1977). 4 leaves/box (12 samples for each species) where collected. Leaf samples were grinded in distilled water, centrifuged at 14000 rpm for 2' and titrated to pH 7 with 0.01N NaOH. Nocturnal malate accumulation of each species at a given date, was expressed as the average value (ml of titrant) of the results of 12 titrations (12 leaves).

2.4.3 Results

The day by day average diurnal water losses (% of the SMC at the start of the experiment) during the experiment, for the vegetated boxes and the bare substrate, are shown in Figure 2.1.4. The average percentage of diurnal water losses, measured as above, on the whole experiment, for the same boxes, are shown in Table 2.4.1.

Table 2.4.1 - Average values and standard deviation of the diurnal water losses (% w/w of the SMC at the start of the experiment) along the whole experiment, for the planted boxes and the bare substrate.

B. erectus	*C. dactylon*	*S. kamtschaticum*	Bare substrate
4.9 ± 1.5	3.8 ± 0.5	3.3 ± 0.8	2.4 ± 1.1
F= 9.60, P = 8.0184 E^{-05}			

By Table 2.4.1, all the planted boxes have an average diurnal water loss higher than the boxes without plants. The average water losses are higher for boxes with *B. erectus*, followed by boxes with *C. dactylon* and *S. kamtschaticum.* Water losses of the boxes with the bare substrate are about half the values recorded for boxes with *B. erectus*. Minor differences are recorded for the boxes vegetated with *C. dactylon* and *S. kamtschaticum* if compared to those with the bare substrate. The boxes planted with *C. dactylon* have an intermediate evapotranspiration activity between those planted with *B. erectus* and those with *S. kamtschaticum*.

Figure 2.4.1 shows the details of the results reported in Table 2.4.1.

B. erectus shown a particular behavior, different from that of *C. dactylon* and *S. kamtschaticum.* Diurnal water losses of *B. erectus* are higher than those of *C. dactylon* and *S. kamtschaticum* till the day 9th. In the first 6 days, *B. erectus* increased its diurnal water losses; from the sixth day onwards the diurnal water losses progressively decreased till the 13th day. *C. dactylon* and *S. kamtschaticum* shown similar diurnal water losses till the 10th day; after this date *S. kamtschaticum* decreased its diurnal water losses, whereas *C. dactylon* maintained nearly unchanged this parameter till the day 16, end of the experiment.

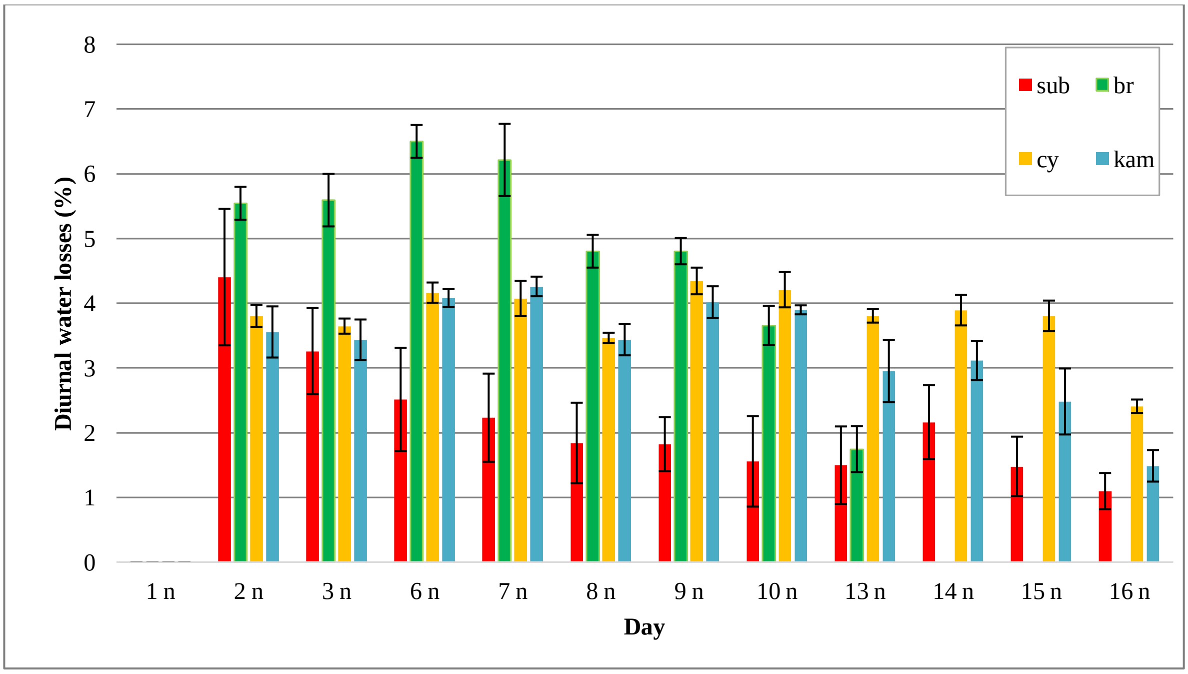

Figure 2.4.1 – Average diurnal water loss (% of the SMC at the start of the experiment) of the vegetated boxes and the bare substrate measured by gravimetric method at the end of the day (n = night, at 7 pm) every 24 hours. The missing days correspond to weekends or other days when the measurements were not done. sub = bare substrate, br = *B. erectus*, cy = *C. dactylon*,, kam = *S. kamtschaticum*..

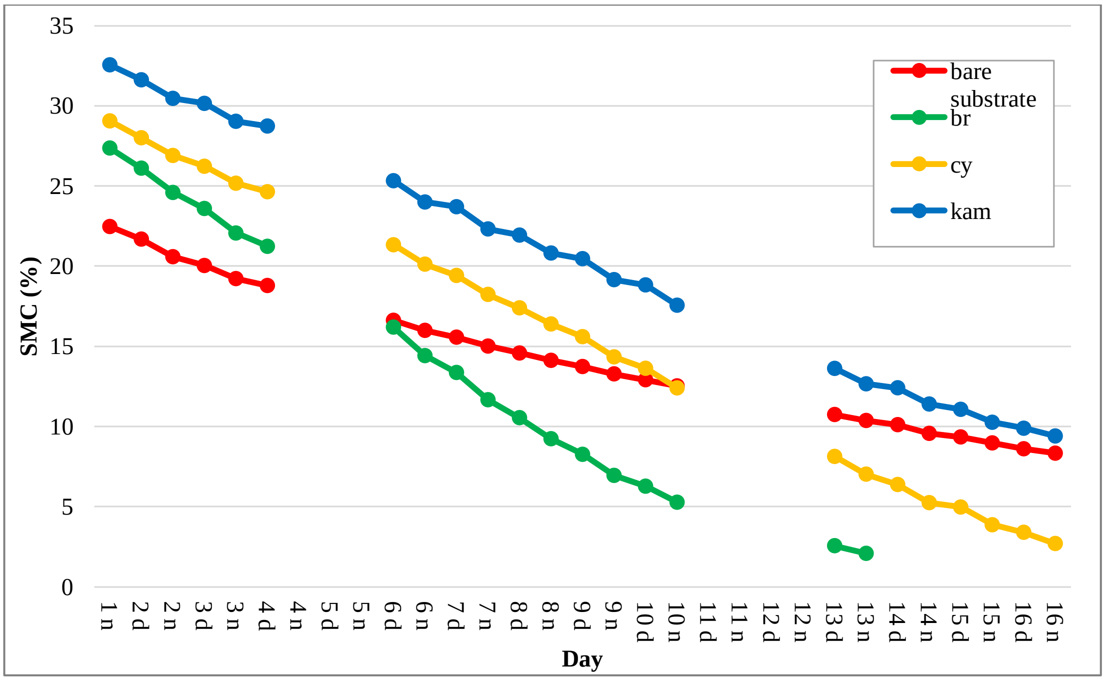

Figure 2.4.2 – Average substrate moisture content (SMC) measured by gravimetric method every 12 hours (d = day, at 7 am and n = night, at 7 pm) in bare substrate and planted boxes. The missing data correspond to weekends or other days when the measurements were not done. br = *B. erectus*, cy = *C. dactylon*,, kam = *S. kamtschaticum.*

The diurnal water loss rates (Figure 2.4.1) and the SMC values (Figure 2.4.2) of the boxes with *B. erectus* showed the highest transpiration activity (diurnal water losses > 4 %) until the 9th day. In particular, boxes with *B. erectus* reached the highest absolute value of diurnal water losses on the 6th day (6.5 %). A decrease was observed from the 10th day, when the substrate reached, on average, 5 % w/w of SMC. *C. dactylon*, instead, maintained diurnal water loss values almost unchanged until the 15th day. These values ranged between 3.5 % and 4.3 % and decreased to 2.4 % on the 16th day when the SMC values were around 2.6 % w/w. The boxes with *S. kamtschaticum* showed the lowest diurnal water losses and a gradual decline of SMC during the whole experiment. Overall (Figure 2.4.2), the SMC of the boxes with *B. erectus* fell quickly (6th day) below the SMC of boxes with bare substrate. The SMC of the boxes with *C. dactylon* fell below the SMC of boxes with bare substrate about at the end of the experiment (13th day). The SMC of the boxes with *S. kamtschaticum* never fell below the SMC of boxes with bare substrate.

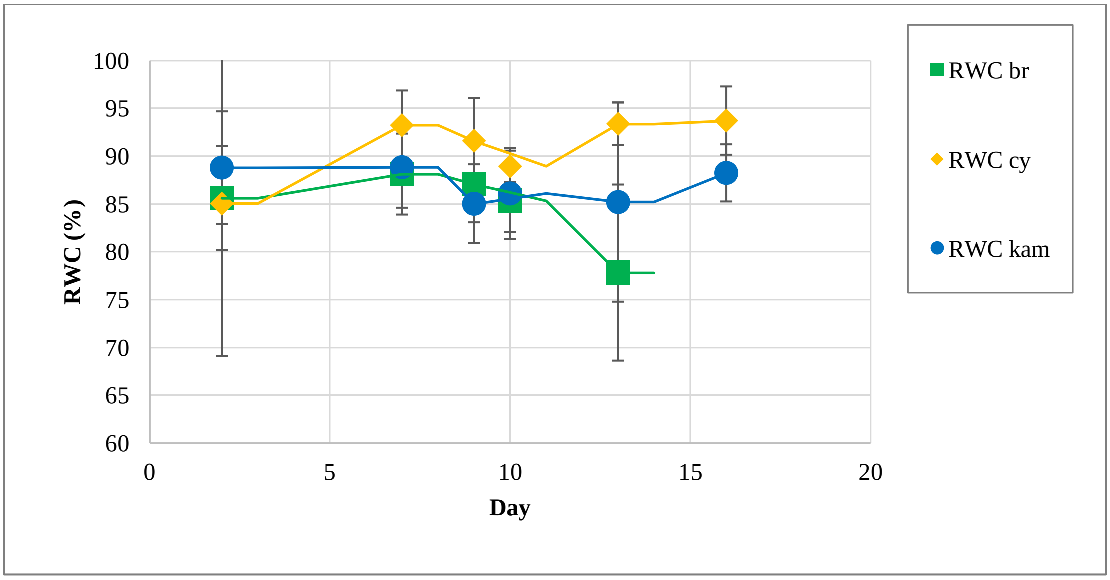

Figure 2.4.3 – Relative Water Content (RWC) ± sd measured at 12 am during the experiment. br = *B. erectus*, cy = *C. dactylon*, kam = *S. kamtschaticum*.

Table 2.4.2 – Average values and standard deviation of the RWC of *B. erectus, C. dactylon, S. kamtschaticum,* during the experiment.

%	Day 2	Day 7	Day 9	Day 10	Day 13	Day 16
B. erectus	85.6 ± 5.4	88.1 ± 4.2	87.1 ± 4.0	85.3 ± 3.3	77.8 ± 9.2	-
C. dactylon	85.0 ± 15.9	93.2 ± 3.6	91.6 ± 4.5	88.9 ± 1.6	93.4 ± 2.2	93.7 ± 3.6
S. kamtschaticum	88.8 ± 5.9	88.8 ± 4.2	85.0 ± 4.1	86.1 ± 4.8	85.2 ± 10.4	88.2 ± 3.0

As far as the RWC of the studied species is concerned, *B. erectus* and *C. dactylon* showed an initial increase of their RWC values (from day 2 to day 7), more evident in *C. dactylon*. In the following days, the RWC values of *B. erectus* fell down evidently, whereas the RWC values of *C. dactylon* oscillated around the 90 % until the day 10 and increased to about 93 % at the day 13 and 16 (end of the experiment). *S. kamtschaticum* maintained its RWC values approximately constant overall the experiment. The RWC rates of *B. erectus* and *S. kamtschaticum* observed in this experiment reflect the trends observed in the first experiment, *Sedum* species showed the highest water saving ability. Regarding to C4 species, the RWC values of *C. dactylon* are always high, and even higher than those of *S. kamtschaticum*, a species low water consuming.

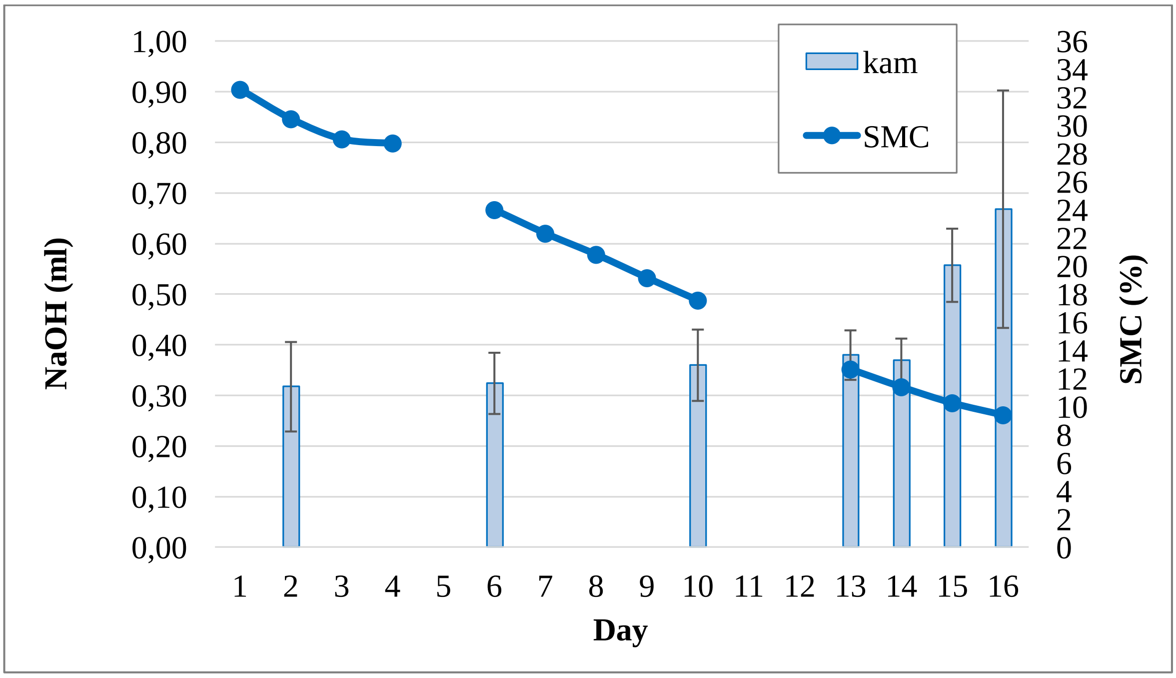

Figure 2.4.4 – Averages values of malate concentration in leaves of *S. kamtschaticum* during the experiment (bars) ± sd. Malate concentration is evaluated as ml of NaOH necessary for the titration. The average values (line) of SMC in the boxes planted with *S. kamtschaticum* is also shown. In the table below is reported also the LSD value.

Table 2.4.3– Averages values of malate concentration in leaves of *S. kamtschaticum* during the experiment (bars) ± sd. Malate concentration is evaluated as ml of NaOH necessary for the titration.

	Day 2	Day 5	Day 9	Day 13	Day 14	Day 15	Day 16
NaOH (ml)	0.32 ± 0.09	0.32 ± 0.06	0.36 ± 0.07	0.38 ± 0.05	0.37 ± 0.04	0.56 ± 0.07	0.67 ± 0.23
F = 17.15, P = 1.76 E^{-12} LSD = 0.09, P = 0.05							

Finally, using the same procedure adopted in the first experiment, the nocturnal malate accumulation in leaves of *S. kamtschaticum* was measured at the days 2, 6, 10, 13, 14, 15, 16 of this experiment. A notable increase of the malate accumulation occurred between the day 14 and the day 15 (Figure 2.4.4 and Table 2.4.3). The corresponding values of SMC at these dates of the experiment were 11 % w/w and 10 % w/w respectively. Comparing these values with those found in the first experiment, the increase of malate in *S. kamtschaticum* was found around 11 % w/w of SMC.

In Table 2.4.4 the diurnal water losses of *B. erectus*, *C. dactylon*, *S. kamtschaticum* and the bare substrate, at different values of SMC, are reported. Different behavior among boxes is evident even at about 20 % w/w of SMC (well-watered conditions). These differences became more evident at about 10 % w/w of SMC when the boxes with *B. erectus* and *C. dactylon* maintained their mean diurnal water losses, already observed at SMC of 20 % w/w, whereas the boxes with *S. kamtschaticum*, because of metabolism change, decreased their water losses (from 3.4 % to 2.5 %). *B. erectus* resulted the species with the highest water losses at both the SMC values of Table 2.4.4.

Table 2.4.4 – Mean diurnal water losses (± sd) of the boxes planted with the studied species and the boxes with bare substrate at different SMC of the substrate. For each value of SMC, results of the ANOVA on the mean diurnal water loss (%) are also reported.

SMC around 20 % w/w (Well-watered condition)		
	Mean diurnal water loss (%)	**Mean diurnal water loss (g)**
bare substrate	3.3 ± 1.1	75.6
B. erectus	5.6 ± 0.4	156.3
C. dactylon	4.2 ± 0.2	124.5
S. kamtschaticum	3.4 ± 0.2	112.9
F = 18.04, P = 0.000641 LSD = 0.8, P = 0.05		
SMC around 10 % w/w (Metabolism change in *S. kamtschaticum*)		
	Mean diurnal water loss (%)	**Mean diurnal water loss (g)**
bare substrate	1.5 ± 0.6	34.4
B. erectus	6.2 ± 0.6	173.0
C. dactylon	4.2 ± 0.3	124.5
S. kamtschaticum	2.5 ± 0.5	112.9
F = 30.66, P = 1.033 E^{-05} LSD = 0.7, P = 0.05		
SMC around 5 % w/w (Comparison between C3 and C4 species)		
	Mean diurnal water loss (%)	**Mean diurnal water loss (g)**
B. erectus	3.7 ± 0.2	103.3
C. dactylon	3.9 ± 0.2	115.6
F = 14.30, P = 0.2978		

Relationships between SMC and diurnal water losses (%) were further investigated by the calculation of the correlation coefficients between the two parameters (Table 2.4.5). All the correlations coefficients are positive and, except for *C. dactylon*, are significantly different from 0, at different level of error probability. For *C. dactylon*, instead, correlation coefficient does not describe a significant dependence relationship between the same parameters.

Table 2.4.5 – Correlation coefficient R and P values between SMC and diurnal water losses (%) for the three studied species and the bare substrate.

	R	**P**
Bare substrate	0.8896 ***	P < 0.001, n = 11
B. erectus	0.6857 *	P < 0.10, n = 8
C. dactylon	0.3455 n.s.	P > 0.10, n=11
S. kamtschaticum	0.6544 **	P < 0.05, n = 11

As might be expected, the best value of the correlation coefficient is that for the bare substrate, corresponding to a linear relation, describing the physical phenomenon of water evaporation. Lower values of the correlation coefficient indicate a control by the plant on the simple physical phenomenon.

Figure 2.4.5 shows the details of the results previously discussed and the functions that best approximate relationship between the two variables.

As far as *B. erectus* is concerned, the relationship among the SMC and diurnal water losses could be better described by a non linear function ($R^2 = 0.7820$ for a power function (Figure 2.4.5) instead of $R^2 = 0.4702$ for a linear function). The function in figure 2.1.8 shows a quickly increasing control on the water losses by *B.erectus*, when the SMC reached very low values (approximately below 7%).

For *S. kamtschaticum* too, the best relationship fitting the experimental points is not linear ($R^2 = 0.5444$), but the R^2 value, corresponding to a linear function, is only slightly lower ($R^2 = 0.4282$). *S. kamtschaticum*, in the range 10 - 15 % w/w of SMC, shows a progressive notable decrease of diurnal water losses. This behavior is congruent with the results obtained from the observations on the malate concentration (at about 10 % w/w of SMC, *Sedum* species switch-on the CAM metabolism).

C. dactylon exerts a particular control on the diurnal water losses. In this species the water losses are practically constant (independent from SMC value) until about 4 % of SMC.

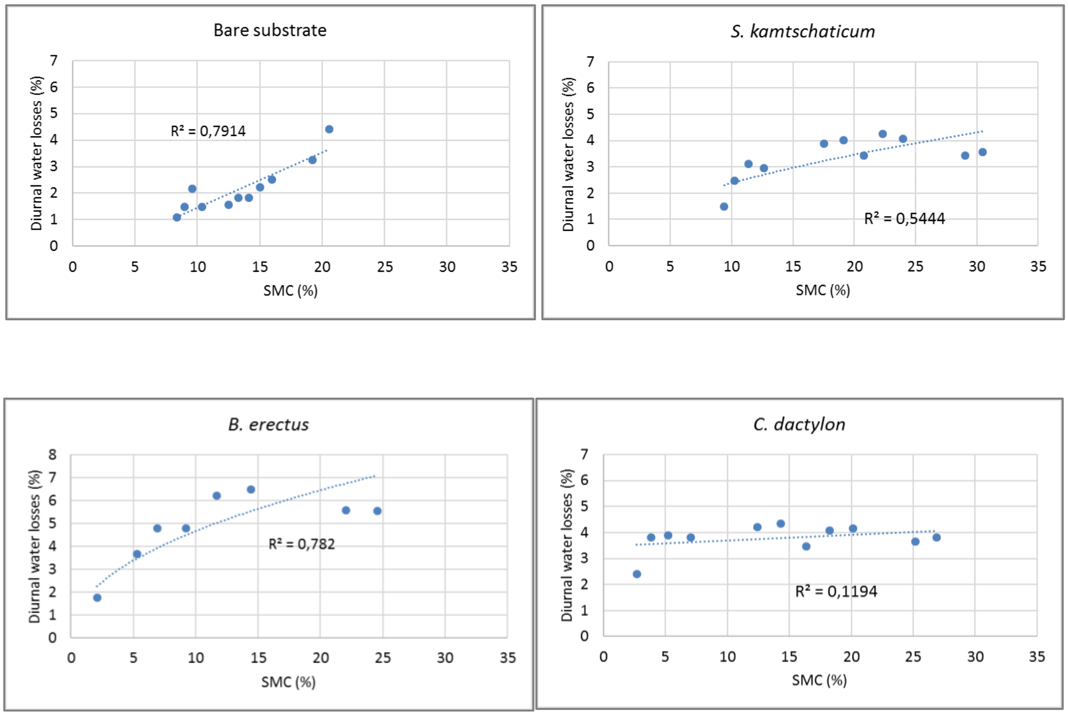

Figure 2.4.5 – Relationships between SMC and diurnal water losses (%) for the three studied species and bare substrate.

2.2.4 Discussion and Conclusions - third experiment

The third experiment has analysed the transpiration of *B. erectus*, *C. dactylon*, S. *kamtschaticum* when the available water decreases, under 28 °C average temperature and other experimental conditions as described in Materials and Methods.

Results obtained confirmed the different behavior of *B. erectus*, a C3 species, if compared with *S. kamtschaticum*, a CAM-facultative species. *B. erectus* continued to transpire even at very low values of SMC, but its RWC reached low values at least three days before the end of the experiment.

S. *kamtschaticum* slowed-down its diurnal transpiration very early, at the 10th – 11th day of the experiment, even at considerable values of SMC (on average, 10 % w/w), in this way, *S. kamtschaticum* maintains high values of RWC during the whole experiment.

The behavior of *C. dactylon*, a C4 species is of particular interest. This species continued to transpire, till the 15th day of the experiment, even at low value of SMC, but maintains good values of RWC, above 85 %, like *S. kamtschaticum.* The water losses are more or less constant along all the experiment till 2.7 % w/w of SMC, unlike for *B. erectus* which initially showed high values of diurnal water loss, but then it also showed a sharp decline strongly dependent on the SMC decrease (Figure 2.4.1).

B. erectus transpiration (and the corresponding cooling effects) are higher than that of *C. dactylon* (and of S. *kamtschaticum*) at up to 5 % w/w of SMC. But, at the final stages of the SMC decreasing, *C. dactylon*, thanks to its capacity to limit the stomatal conductance with low effect on the photosynthesis, could provide, depending on the duration of water stress (Ghannoum, 2008), a positive cooling effect. Thus, *C. dactylon* could be a valid choice to reduce the irrigation frequencies, but providing, at the same time, a better cooling effect if compared to that produced by *Sedum* species.

2.5 FOURTH EXPERIMENT: The green roof’s thermal performance during the cold season: first results from different plant covers.

2.5.1 Introduction and Objective

Green roof plant cover plays an important role concerning insulation from low external during the winter season. (Lundholm *et al*, 2014; Eksi *et al*., 2017).

The fourth experiment was planned in order to accomplish the second objective of the thesis: to evaluate the winter insulation ability of the same group of species (*B. erectus*, *C. dactylon*, *L. corniculatus*, *S. lydium*, *S. kamtschaticum*), whose summer cooling performances were previously evaluated.

2.5.2 Material and Methods

Six boxes were utilized (40 cm x 30 cm x 22 cm height), each filled with 1500 g of green roof's substrate (Harpo/SEIC substrate) with the Harpo commercial stratification for extensive green roofs. On September 2016, five boxes were planted, each with one species (*B. erectus*, *L. corniculatus*, *C. dactylon*, *S. lydium*, *S. kamtschaticum*) at high density, to obtain a complete substrate cover (100 % of coverage) and they were left to grow outside. The sixth box was filled only with bare substrate, as control.

At the start of the experiment, on 18 January 2017, the tested species were at winter vegetative pause and their cover was as in Figure 2.5.1. In particular:

- *B. erectus*, created many compact yellow tufts about 30 cm high that provided a 100 % coverage of the box surface.
- *L. corniculatus* and *C. dactylon*, instead, lost their leaves, so their branches did not completely cover the substrate.
- *S. lydium and S. kamtschaticum*, susceptible to freezing injury, as reported by Benvenuti and Bacci (2010), show some spaces in the compact and thin (less than 5 cm) plant layer.

Each box, was equipped with two thermistors (Thermistor Probe Temperature Data Logger, OM-EL-USB-TP-LCD, probe type K, Omega, IT. Accuracy: ± 0.1 °C, resolution: ± 0.5 °C): one buried into the substrate at 7 cm depth, to collect the internal temperatures and another one, placed on the substrate under the plant cover (on the bare substrate, for the control box) to collect the surface temperatures. The experiment lasted 50 days, from 18th January 2017 to 8th March 2017.

The datalogger of each thermistor recorded the temperature values every 30 minutes. The mean temperature between 4:45 am and 7:45 am (mean of seven recorded values), the coldest period of the day, was used to assess the insulation ability of each species, day by day.

We also collected the values of the daily minimum temperature, recorded by a meteorological station placed in the outside area of DipSA (44°33’ 03” N, 11°24’ 36” E, 33 m a.s.l. European Datum 1950, UTM 32), not far from the experimental boxes.

Figure 2.5.1 – The six boxes monitored during the experiment. 1 = Bare substrate, 2 = *B. erectus*, 3 = *L. corniculatus*, 4 = *C. dactylon*, 5 = *S. lydium*, 6 = *S. kamtschaticum*.

2.5.3 Results

The values of the daily minimum temperature, recorded on the surface of the bare substrate, and the values of the daily minimum temperature, allow to recognize three different periods during the experiment (Figure 2.5.2 and Table 2.5.1; Annexes 5, 6). The first one, from 18th January to 30th January (day 1 to day 13), is characterized by a minimum average surface temperature of -0.8 °C, and of -1.4 °C in the atmosphere. The second period, from 31th January to 26th February (day 14 to day 40), is characterized by a minimum average surface temperature of 2.9 °C and a minimum average atmospheric temperature of 3.6 °C. In the third period, from 27th February to 8th March (day 40 to day 50), the temperatures increased further; the minimum average surface temperature was 5.2 °C and a minimum average atmospheric temperature 6.1 °C. In the first period, the daily minimum temperature frequently fell below 0 °C, in the second and third period the minimum daily temperature rarely fell below 0 °C.

The insulation ability of each species was estimated by comparing the depth temperatures (at -7 cm), recorded in each of the five vegetated boxes, and the depth temperature of the box with bare substrate only (Table 2.5.2).

Table 2.5.2 –Average minimum depth temperatures, average minimum surface temperatures, thermal contribution from the bare substrate (average depth temperatures minus average surface temperatures) and thermal contribution from the plant cover (average depth temperatures of each species minus average depth temperatures of bare substrate) on the average minimum depth temperatures ± standard deviation, in three period of the experiment. For each period, results of ANOVA, performed on the average minimum depth temperatures, on the average minimum surface temperatures and on thermal contribution from the plant cover on the average minimum depth temperatures are indicated.

1st Period: day 1 to day 13				
	Average minimum depth temperatures (°C)	**Average minimum surface temperatures (°C)**	**Thermal contribution from the bare substrate on the average minimum depth temperatures (°C)**	**Thermal contribution from the plant cover on the average minimum depth temperatures (°C)**
Bare substrate	**-0.2 ± 0.3**	-0.8 ± 1.0	0.6 ± 0.9	//
B. erectus	0.4 ± 0.4	-0.2 ± 0.9		0.6 ± 0.2
L. corniculatus	0.1 ± 0.3	-0.1 ± 0.4		0.4 ± 0.2
C. dactylon	0.0 ± 0.2	-0.5 ± 1.0		0.2 ± 0.2
S. kamtschaticum	0.2 ± 0.5	-0.5 ± 1.4		0.5 ± 0.4
S. lydium	0.1 ± 0.1	-0.4 ± 0.7		0.3 ± 0.3
LSD (P = 0.05)	0.2	//		0.2
ANOVA	F = 5.8536 P = 0.0001	F = 0.8941 P = 0.4898		F = 3.2884 P = 0.0167

During the first period of the experiment (Table 2.5.2), the average minimum depth temperatures ranged from -0.2 °C in the box with the bare substrate, to 0.4 °C in the box with *B. erectus*. Standard deviations on the average minimum depth temperatures ranged from 0.1 °C (*S. lydium*) to 0.5 °C (*S. kamtschaticum*). The average minimum surface temperatures ranged from -0.8 °C in the box with the bare substrate, to -0.1 °C in the box with *L. corniculatus*. Standard deviations on the average minimum surface temperatures, ranged from 1.4 °C (*S. kamtschaticum*) to 0.4 °C (*L. corniculatus*). The ranges of variation of the average minimum depth temperatures (0.6 °C) was not so different from the range of variation of the average minimum surface temperatures (0.7 °C); more wide were, instead, the ranges of variation of the values of the standard deviations (0.4 °C and 1.0 °C, respectively). Results of ANOVA, performed on the average minimum depth temperatures, indicate that the average minimum depth temperatures of all the vegetated boxes are higher than that of the box with the bare substrate. As far as the mitigation of the depth temperatures by the plant cover is concerned, the best performance is that of *B. erectus* (thermal contribution from the plant cover equal to 0.6 °C), followed by that of *S. kamtschaticum* (thermal contribution from the plant cover equal to 0.5 °C). The worst performance was, instead, that of *C. dactylon* (thermal contribution from the plant cover equal to 0.2 °C). Results of ANOVA, performed on the values of the thermal contribution from the plant cover, indicate that there are significant differences between species.

Results of ANOVA, performed on the average minimum surface temperatures, indicate that the average minimum surface temperatures of all the vegetated boxes are not significantly different from that of the box with the bare substrate.

In conclusion, during the first period of the experiment, the coldest one, the thermal contribution from the bare substrate on the average minimum depth temperatures was, on average, 0.6 ± 0.9 °C. Significant mitigation effects due to the plant cover, were observed only on the deep temperatures. *B. erectus* performs significantly better than the other studied species.

2nd Period: (day 14 to day 40)				
	Average minimum depth temperatures (°C)	**Average minimum surface temperatures (°C)**	**Thermal contribution from the bare substrate on the average minimum depth temperature (°C)**	**Thermal contribution from the plant cover on the average minimum depth temperature (°C)**
Bare substrate	**4.0 ± 1.8**	2.9 ± 2.3	1.0 ± 0.9	//
B. erectus	5.4 ± 1.5	4.4 ± 1.7		1.5 ± 0.8
L. corniculatus	5.0 ± 1.6	4.2 ± 1.8		1.0 ± 0.6
C. dactylon	4.8 ± 1.7	3.8 ± 2.0		0.8 ± 0.3
S. kamtschaticum	5.1 ± 1.5	3.8 ± 1.9		1.1 ± 0.7
S. lydium	4.8 ± 1.6	4.0 ± 1.8		0.9 ± 0.4
LSD (P = 0.05)	0.9	//		0.5
ANOVA	F = 2.4722 P = 0.0347	F = 1.8027 P = 0.1154		F = 45.4745 P = $8.5397\ E^{-29}$

During the second period of the experiment (Table 2.5.2), the average minimum depth temperatures ranged from 4.0 °C in the box with the bare substrate, to 5.4 °C in the box with *B. erectus.* Standard deviations on the average minimum depth temperatures ranged from 1.5 °C (*B. erectus* and *S. kamtschaticum*) to 1.8 °C (bare substrate). The average minimum surface temperatures ranged from 2.9 °C in the box with the bare substrate, to 4.4 °C in the box with *B. erectus*. Standard deviations on the average minimum surface temperatures, ranged from 1.7 °C (*B. erectus*) to 2.3 °C (bare substrate). The ranges of variation of the average minimum depth temperatures (1.4 °C) was not so different from the range of variation of the average minimum surface temperatures (1.5 °C); more wide were, instead, the ranges of variation of the values of the standard deviations (0.4 °C and 0.6 °C, respectively).

Results of ANOVA, performed on the average minimum depth temperatures, indicate that the average minimum depth temperatures of all the vegetated boxes are higher than that of the box with the bare substrate, with the exception of that vegetated with *Cynodon dactylon* and *S. lydium*. Not significant differences between species were observed. As far as the mitigation of the depth temperatures by the plant cover is concerned (shown in the last column of the Table 2.5.2) , the best performance was that of *B. erectus* (thermal contribution from the plant cover equal to 1.5 °C), followed by that of *S. kamtschaticum* (thermal contribution from the plant cover equal to 1.1 °C). The worst performance was, instead, that of *C. dactylon* and *S. lydium* (thermal contribution from the plant cover equal to 0.8-0.9 °C). These average values, however, are not so different among them.

Results of ANOVA, performed on the average minimum surface temperatures, indicate that there are not significant differences between the boxes.

In conclusion, during the second period of the experiment, when the atmospheric temperatures started to increase, the thermal contribution from the bare substrate on the average minimum depth temperatures increased, on average, to 1.0 ± 0.9 °C. Significant mitigation effects due to the plant cover, were observed only on the deep temperatures, but not for all the studied species (not for *C. dactylon* and *S. lydium*).

3rd Period: (day 40 to day 50)				
	Average minimum depth temperatures (°C)	**Average minimum surface temperatures (°C)**	**Thermal contribution from the bare substrate on the average minimum depth temperature (°C)**	**Thermal contribution from the plant cover on the average minimum depth temperature (°C)**
Bare substrate	**6.4 ± 2.0**	5.2 ± 2.9	1.2 ± 1.4	//
B. erectus	8.4 ± 1.5	6.8 ± 2.2		2.0 ± 0.7
L. corniculatus	7.9 ± 1.7	6.5 ± 2.1		1.5 ± 0.6
C. dactylon	7.4 ± 1.8	6.1 ± 2.6		1.0 ± 0.3
S. kamtschaticum	7.9 ± 1.7	6.3 ± 2.6		1.5 ± 0.5
S. lydium	7.3 ± 1.8	6.3 ± 2.3		0.9 ± 0.3
LSD (P = 0.05)	//	//		0.7
ANOVA	F = 1.5013 P = 0.2048	F = 0.5239 P = 0.7571		F = 2.8240 P = 0.0245

During the third period of the experiment (Table 2.5.2), the average minimum depth temperatures ranged from 6.4 °C in the box with the bare substrate, to 8.4 °C in the box with *B. erectus.* Standard deviations on the average minimum depth temperatures ranged from 1.5 °C (*B. erectus*) to 2.0 °C (bare substrate). The average minimum surface temperatures ranged from 5.2 °C in the box with the bare substrate, to 6.8 °C in the box with *B. erectus*. Standard deviations on the average minimum surface temperatures, ranged from 1.5 °C (*B. erectus*) to 2.0 °C (*bare substrate*). The ranges of variation of the average minimum depth temperatures (2.0 °C) was not so different from the range of variation of the average minimum surface temperatures (1.8 °C); more wide were, instead, the ranges of variation of the values of the standard deviations (0.5 °C and 0.8 °C, respectively).

Results of ANOVA, performed on the average minimum depth temperatures, indicate that the differences are not significant, only the average minimum depth temperature of the box vegetated with *B. erectus* was higher than that of the box with the bare substrate (F = 6.0519, P = 0.02, this ANOVA values not shown in the Table 2.5.2). As far as the mitigation of the depth temperatures by the plant cover is concerned, the best performance of *B. erectus*, is confirmed its thermal contribution (equal to 2.0 °C) is the higher followed by that of *L. corniculatus* and *S. kamtschaticum* (thermal contribution from the plant cover equal to 1.5 °C). The worst performance was, instead, that of *S. lydium* (thermal contribution from the plant cover equal to 0.9 °C). These values, however, are not so different than the contribution of bare substrate (except for *B. erectus*).

Results of ANOVA, performed on the average minimum surface temperatures, indicate that the average minimum surface temperatures of all the vegetated boxes are not significantly different from that of the box with the bare substrate.

Thus, during the third period of the experiment, when the atmospheric temperatures further increased, the thermal contribution from the bare substrate on the average minimum depth temperatures showed, indicatively, only a weak increase to 1.2 °C. Significant mitigation effects due to the plant cover were observed only on the box with *B. erectus* (depth temperatures, on average, 2.0 °C more than the average depth temperature of bare substrate).

In conclusion, during the whole experiment, the thermal contribution of the plant cover varied, on average, from 0.2 to 0.6 °C in the first period, on average, from 0.8 to 1.5 °C during the second one while in the last period, the thermal contribution varied, on average, from 0.9 to 2.0 °C. These contributions were higher than the thermal contribution of the substrate that, in the same periods, varied, on average, from 0.6, to 1.0, to 1.2 °C.

2.5.4 **Discussion and Conclusions – fourth experiment**

The experiment compared the insulation ability of herbaceous species that, during the winter, have a different development and quantity of leaves and ramifications. The experiment examined, further, the insulating effect of a commercial substrate for green roofs.

The plant cover has always exerted an effect of mitigation of the external temperatures on the deep temperatures of the substrate. In the vegetated boxes, the deep temperatures are always higher than the deep temperatures of the box with the bare substrate.

During the first period of the experiment, the coldest one, the average deep temperatures of the vegetated boxes never fell below zero. Plant covers showed a positive effect on the mitigation of the external temperatures and their average depth temperatures were often significantly higher than those of box with bare substrate, in particular during the second period. Regarding the species tested, *B. erectus* coverage showed the best insulation contribute and it is also the only species that showed significant differences, as compared to the bare substrate, during all periods of experiment. The better performance of *B. erectus* was reasonably due to the higher amount of biomass produced during the vegetative season and permanent during the winter, which better acts as insulating layer during the cold season. *L. corniculatus* and *C. dactylon* lost their leaves in winter and *Sedum* species showed some freezing injury that reduced its coverage.

However, Lundholm *et al*. (2014) explained that an abundant plant cover due, for example, to high LAI values had a negative effect because of the reduction in solar heat gain under the vegetation. Vegetation types that reduce the temperature drop overnight could have negative effect on the heating of the substrate during the sunny days. In this case, green roofs planted with *Sedum* species or without plants, could increase more rapidly the temperature of substrate layer than green roof with *B. erectus*.

This behavior explains the gain in the thermal insulation provided by the plant layer (especially by *B. erectus*) to the increase in the external temperature during the experiment.

When the external temperatures dropped below zero during the night, substrate began to lose heat and the plants played an important role to reduce this loss. On the other hands, during the day, when the sun heats up the surface, plant canopy reduced the solar heat gain and, consequently, the heat accumulated in the substrate before the next night. Thus, the substrate above plant canopy (in particular above *B. erectus*) needed more time to warm up. When the external temperatures increased, the negative effect of the plant cover began less important than the positive effect provided during the night.

2.6 CONCLUSIONS

According to the results obtained from the indoor and outdoor experiments reported in the second chapter, the use of xerophilus or semi-xerophilus C3 and C4 species on green roofs, as an alternative to the usual Crassulaceae species (like *Sedum* species), is possible and, also, could provide significant advantages as far as the thermal property of rooftops, both in summer and winter season, are concerned.

The first three experiments confirmed that C3 (*B. erectus*, *L. corniculatus*, and *S. officinalis*) and C4 species tested (*C. dactylon*) offer better performances than the CAM-facultative species (*S. kamtscahticum* and *S. lydium*), when summer cooling effects are required. Better performances of the C3 and C4 species were observed both under well-watered conditions and under low substrate moisture, when *Sedum* species began to use CAM metabolism.

The critical value of soil or substrate moisture content (SMC), inducing the switch-on of the CAM metabolism in CAM-facultative species, was identified, empirically, through the observations of nocturnal malate accumulation in the *Sedum* leaves, associated to diurnal water loss rate (in the first and third experiments) and through the gas exchange activity (in the second experiment). It has been identified at between 11 % and 6 % w/w in the first and third experiments and at around 12 % v/v through the observations of gas exchange activities, in the second experiment.

In C4 species (*C. dactylon*) diurnal water losses remained almost unchanged from the start of the experiment, when SMC was high, until very critical SMC values (5 % w/w). The amount of diurnal water losses in *C. dactylon* was intermediate between that of *Sedum* and the C3 species. In addition, this diurnal amount of water was transpired over a longer period than for any other studied species. Thus, C4 plants could provide a more durable cooling effect than the C3 species, at equal SMC values.

The second experiment pointed out that the cooling capacity was also positively influenced by other factors, such as the leaf morphology, LAI and in particular by the thickness of canopy (covering ability) (Kumar and Kaushik, 2005; Eksi *et al*., 2017).

The winter experiment demonstrated that the plant cover could give an important positive contribution to the thermal performance of the green roofs, even during the cold season. The different contributions observed by various species depended on the amount of biomass produced during the previous growing season and maintaining this biomass during the winter season.

Among the studied species, *B. erectus* maintained the higher biomass during the whole winter experiment, allowing a better thermal insulation than the bare substrate or than the other species, by being 2 °C warmer at 7 cm substrate depth. *C. dactylon* and *L. corniculatus*, two species with good cooling capacity during the hot season, maintained instead a low biomass in winter, insufficient to guarantee an efficient insulation. During the winter experiment, in fact, the boxes planted with *C. dactylon* and *L. corniculatus* showed sometimes lower depth temperatures than those recorded in boxes planted with *Sedum* species.

3. THERMAL PERFORMANCES OF PLANT SPECIES

3.1 Growth and spread of native perennial herbaceous species on a green roof

3.1.1 Introduction and Objectives

Many studies investigated on the use and the survival of native species on different green roof's typologies (Monterusso *et al.*, 2005; Blanusa *et al*. 2013; Vestrella *et al.*, 2015). Even if native *taxa* have a wide potential of utilization on green roofs, thanks to their adaptation to the local climates, experimental results are sometime contradictory. For example, Monterusso *et al*. (2005) tested native grassland perennials on non-irrigated extensive green roofs, using 10 cm of growing medium and only 4 out of 18 survived after three growing seasons. The environment of an extensive green roof does not often match with the environmental requirements of the used native species and/or their seeds do not easily germinate on rooftops (White and Snodgrass, 2003). A specific experimentation become necessary to increase the chances of success. On the other hand, native species can provide greater biodiversity than a typical monoculture of non-native *Sedum* species (Maclvor *et al.*, 2011; Williams *et al.*, 2014; Vestrella *et al.*, 2015) and a more efficient cooling effect, due to their C3 metabolism or to the shape and size of leaves (Blanusa *et al*. 2013).

Although the strong positive relationship between plant biodiversity and ecosystem functioning has been well-established in the ecological literature (Hooper *et al.*, 2005; Cook-Patton and Bauerle, 2012), the empirical research linking plant biodiversity with green roof performance is limited. Consequently, the green roof designers infrequently use native species rather than the *Sedum* monoculture (Cook-Patton and Bauerle, 2012).

The following article (accepted in special issue of Acta Horticulturae IRHS, “Greener Cities 2017”) aimed to test the survival and the growth capacity of 6 wild herbaceous perennial species (*Festuca ovina* L., *Thymus serpyllum* L., *Hieracium pilosella* L., *Acinos alpinus* (L.) Moench, *Sanguisorba minor* Scop. and *Achillea millefolium* L.). They are common of the arid and semi-arid grassland communities (Xerobromion and Mesobromion communities) of the Apennines, and they were tested on a green roof of the School of Agriculture of Bologna, under a subcontinental temperate climate, with hot summers and moderately cold winters, and a low irrigation and fertilization management.

All plant material was collected in natural and semi-natural grassland communities of the Emilian Apennines, cultivated and reproduced in a nursery at the Azienda Agraria of the Bologna University (AUB).

3.1.2 Growth and spread of native perennial herbaceous species on a green roof

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Abstract

The European Communication on Green Infrastructures encourages the use of wildflower verges, green walls, green roofs, etc. to mitigate the artificiality of the urban and rural environment. Green roofs are engineered ecosystems occupying underutilized urban spaces that rely on the plant cover to provide services, such as the reduction of temperature, retention of storm-water and enhancement of urban biodiversity. In this framework we explored the ability of some wild perennial species of arid and semi-arid grasslands to survive and colonize the substrate of the extensive green roofs of the Agricultural Sciences School of the University of Bologna. In mid-June 2015 young plants of *Festuca ovina* L., *Thymus serpyllum* L., *Hieracium pilosella* L., *Acinos alpinus* (L.) Moench, *Sanguisorba minor* Scop. and *Achillea millefolium* L, coming from wild local populations, were transplanted into containers (54x54x9 cm depth) forming the upper cover of a green roof. The plants were fertilized and then irrigated throughout the summer of 2015. The growth of the species (coverage and space occupation) was monitored approximately once a month, from August 2015 to May 2016. *F. ovina* reached the highest values of coverage, without any significant seasonal variations. *T. serpyllum* and *H. pilosella* reached moderate coverage values depending however on the seasons. *A. millefolium* showed very effective dispersal abilities, but poor coverage potential. It can be used as a filler species in multi-species green roofs. Our results showed that the wild local flora can be an important, though still poorly explored, reserve of biodiversity for a new generation of extensive green roofs, designed, following a careful selection of species, for the best possible performances of the services they provide.

Keywords: Green Infrastructures (GI), Extensive Green Roofs (EGR), wild species, urban biodiversity, ecosystem services

Introduction

The European Communication on Green Infrastructures and the European Strategy on Green Infrastructures (COM, 2013), as well as the associated planned network of natural and semi-natural areas, aim to improve environmental conditions, citizens' health and quality of life in the European territory. They also support green economy, create job opportunities and enhance biodiversity.

Since green spaces in cities are becoming progressively reduced, roofs, which can reach up to 32 % of the horizontal surface of the built-up areas (Frazer, 2005), have been identified as important underused places where plant cover can be developed (Franzaring *et al*., 2016). In Germany, in the early 21st century, the diffusion of green roofs increased by approximately 13.5 million square meters per year (Oberndorfer *et al*., 2007).

The potential environmental benefits of green roofs are numerous (Del Barrio, 1998; Köhler, 2003; Porsche and Köhler, 2003; Dunnett and Kingsbury, 2004; Oberndorfer *et al*., 2007; Williams *et al*., 2014; Franzaring *et al*., 2016). However, till now green roofs have been constructed with attention paid mainly to the architectural and engineering problems and much less to the quality, performances and environmental congruence of the plant cover, as well as to the possibilities offered by the utilization of the wild species of the local flora. These environmental joint values have only recently begun to be considered (Cook-Patton *et* *al*., 2012) in the realization of green roofs, experimenting with plant covers alternative to those most frequently adopted, commonly involving a mixture of *Sedum* species (Monterusso *et al*., 2005; Maclvor *et al*., 2011; Blanusa *et al*. 2013; Williams et al., 2014; Vestrella *et al*., 2015).

Results are sometimes contradictory (White and Snodgrass, 2003; Monterusso *et al*., 2005) and further experiments are necessary to tune the utilization of native species for green roof cover, maintaining the environmental services they usually supply (Brenneisen, 2006; Lundholm, 2006; Maclvor *et al*., 2011).

The current study aims to test the survival, growth and ways of space occupation of six wild herbaceous perennial species, from arid and semi-arid temperate grassland communities *(Xerobromion* and *Mesobromion* communities) planted on an extensive green roof in the city of Bologna, with a commercial stratification. Given the habitat where these species naturally occur, they appear potentially interesting for the plant cover of green roofs subjected to a climate of sub-Mediterranean type, with hot and moderately arid summers, humid and moderately cold winters, and managed with minimum irrigation and fertilization.

Materials and Methods

The green roof design

The green roof (Annex 7) where the experiment was performed has a surface of 12 x 5 m and covers a low building of the Agricultural Sciences School of the Bologna University (Bologna, Italy). The stratification adopted has a total depth of about 18 cm, where the upper part is organized in quadrate modules of 54 x 54 x 9 cm depth, made of regenerated plastic, interlocking each other and filled with pumice grains (Ø 3-6 mm) and with VULCAFLOR® up to the brim. The surface of each module is covered with 2 cm depth of white pebble gravel as mulching. The bottom part of the stratification, lying under the modular system at a depth of about 9 cm, consists of a system of bags which the roots of plants can penetrate, filled with perlite grains and functioning as a water reserve. The green roof area used in this study comprises 60 modules arranged in 10 rows, each with 6 modules.

The plant species

We selected six herbaceous perennial species coming from wild local populations of arid and semi-arid grassland communities (*Xerobromion* and *Mesobromion* communities). The wild plant material was propagated and maintained under cultivation in a nursery for almost one year before planting on the green roof. In particular the tested species were: *Sanguisorba minor* Scop. (Rosaceae), *Thymus serpyllum* L. and *Acinos alpinus* (L.) Moench (Lamiaceae), *Achillea millefolium* L. and *Hieracium pilosella* L. (Asteraceae), *Festuca ovina* L. (Gramineae). All are frequent on well-drained soils and are drought tolerant, but have different ways of growth, expansion and available space occupation.

As far as the latter aspect is concerned, the studied species can be distinguished into two main groups (Table 1). The species of the first group colonize the available space by forming a continuous and compact cover. This group includes *S. minor*, a perennial medium size forb with a basal rosette of compound leaves, which expands uniformly during spring-summer (Sydes and Grime, 1984), *A. alpinus* which creates very small bushes, and *F. ovina*, a perennial caespitose small tussock-forming grass, that grows in tufts. The second group comprehends stoloniferous creeping species that quickly spread horizontally through stolons (*T. serpyllum* and *H. pilosella*), or more slowly through rhizomes, such as *A. millefolium*, where an efficient sexual reproduction coexists with the vegetative propagation (Warwick and Black, 1982). The species of the second group colonize the available space by forming a discontinuous cover, with many empty patches.

Table 1. - Habitus of the studied species, parameters and number of samples monitored.

Species	Habitus	Parameters observed	N. of samples
S. minor	Basal rosette	Rosette area (cm^2)	3 rosettes/module x 6 modules
A. alpinus	Bushy	Branch length (cm)	6 branches/module x 12 modules
F. ovina	Caespitose	Number of shoots	3 tufts/module x 18 modules
T. serpyllum	Creeping	Stolon length (cm)	6 stolons/module x 12 modules
H. pilosella	Stoloniferous	Stolon length (cm)	6 stolons/module x 6 modules
A. millefolium	Rhizomatous	N. of new shoots per rhizome	5 rhizomes/module x 6 modules

Planting and growth monitoring

In mid June 2015, each of the 60 modules of the green roof was planted with a given number of individuals, depending on the species considered. In detail, we planted 5 plants of *S. minor*/module, in 6 modules, 5 plants of *A. alpinus*/module in 12 modules, 32 shoots of *F. ovina*/module in 18 modules, 5 plants (1 cm rhizome) of *A. millefolium*/module in 6 modules; 4 plants of *T. serpyllum*/module in 12 modules and 4 plants of *H. pilosella*/module in 6 modules. At the start of the experiment each species covered a very low percentage of the available space of each module (from 0.5 % to ≤ 5 %).

The monitoring period started in August 2015 and finished in May 2016. From August 2015 to October 2015 and from February 2016 to May 2016, we monitored the plants' growth once a month. In order to better describe the behavior of each species in colonizing the available space of the modules, different morphological parameters were monitored (Table1), depending on the species and its particular way of growth.

The plant species coverage was estimated through the procedure proposed by Maclvor and Lundholm (2011), putting a digital pin-frame subdivided into 25 rectangular areas of 10.8 cm x 10.8 cm, each with a point (6 mm diameter) on the photo of each module. Percent cover (%) was recorded as the number of points touched by any part of the plants growing in each module (leaves and dead branches were excluded) divided by 25. The percent cover of a given species at a given date is the average of the different values recorded at this date on all the monitored modules.

Irrigation and fertilization management

The green roof is equipped with underground irrigation and sprinkling irrigation with 2 rotors (Rain Bird, 3500 series rotors, 0.12 m^3/h, Azusa, California, USA). After planting in mid June 2015 and till the 30th September 2015, the green roof was irrigated with sprinkler irrigation for 12 minutes, three times per week (6.5 mm per week).

All plants were fertilized with a slow release fertilizer, Nitrophoska®, on 20 June 2015, just after planting. Different amounts of fertilizer were used depending on the number of plants per module (8.96 g/m^2 for *F. ovina*; 1.40 g/m^2 for each module of *A. millefolium, A. alpinus* and *S. minor*; 1.12 g/m^2 for the module of *T. serpyllum* and *H. pilosella*).

Climatic and meteorological characterization

Bologna has a warm humid temperate climate with hot and moderately dry summers; the total annual precipitation, even though quite variable, is on average 750 mm (Ventura et al., 2002, Matzneller et al., 2010).

During the months of the experiment (Table 2) the mean temperature was 17 °C; the minimum average monthly temperature was 4.1 °C in January 2016 (minimum absolute temperature -6 °C on January 19, 2016), the maximum average monthly temperature was 28.8 °C in July 2015 (maximum absolute temperature was 40°C on July 22 and 23, 2015).-The total precipitation was 619.4 mm; the driest month was July 2015 (0.8 mm), whereas October 2015 and February 2016 were the months with the highest precipitation (113.4 mm and 147.6 mm, respectively).

Table 2. - Average monthly temperature (T) and total monthly precipitation (P) during the period of the experiment (June 2015 – May 2016). Data were collected at the agrometeorological station at the University of Bologna experimental farm of Cadriano (44°33’ 03” N, 11°24’ 36” E, 33 m a.s.l. European Datum 1950, UTM 32).

	June	**July**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**
T (°C)	23.4	28.8	25.7	20.3	13.6	9.0	4.8	4.1	7.3	9.8	15.2	18.1
P (mm)	46.8	0.8	34.8	15.8	113.4	52.2	4.6	26.8	147.6	59.2	45.0	72.4

Statistical analyses

Statistical analyses were performed by R free software. We used ANOVA test and Tukey test to identify significant differences among species, for cover or other growth parameters, considering the overall monitored period, as well as significant differences between months for cover or other growth parameters of a given species.

Results

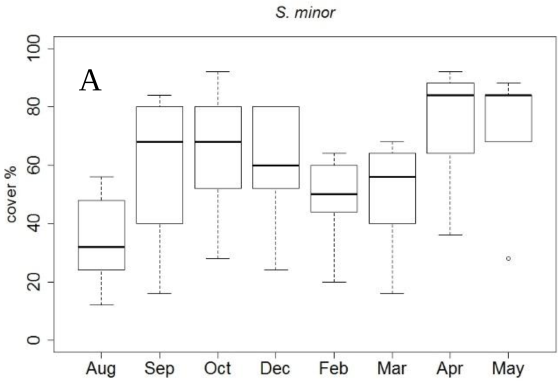
General considerations

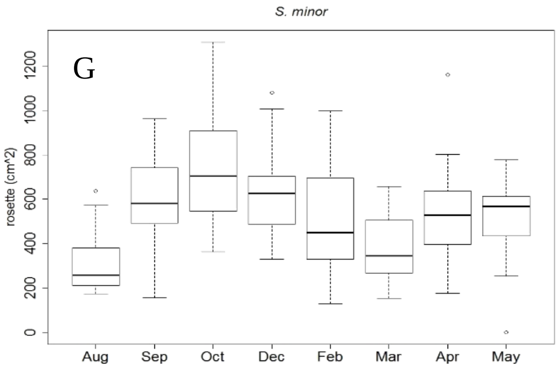
Table 3 shows some general data on the percent cover of the six studied species over the whole period of observation. All the species increased their cover percentage. The greatest average values of cover at the end of the observation period were those of *S. minor* (73 %) and *F. ovina* (69 %). *H. pilosella* (47 %), *T. serpyllum* (38 %) and *A. millefolium* (38 %) reached moderate average cover values, whereas *A. alpinus* reached only a modest average value (19 %). *F. ovina* increased its percent cover by six times, passing from a starting cover of 10% to a final cover of 60 %. *S. minor* duplicated its starting cover. The other species increased their initial cover by less than two times.

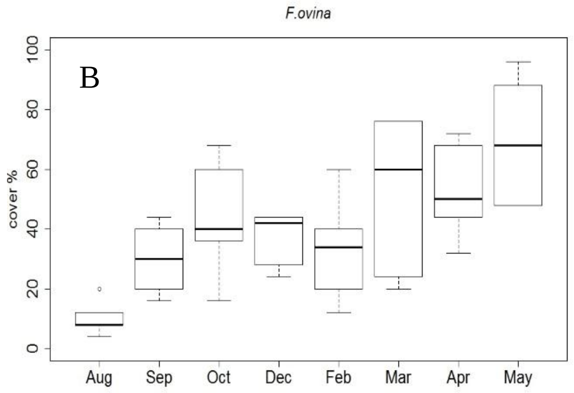
At the end of the observation period, in May 2016, three of the studied species (*A. alpinus, S. minor, T. serpyllum*) started to reduce their cover values, probably as a response to the increase in temperature and the decrease in water availability. We can note that the standard deviation and the variation coefficient values of the considered parameters are always quite high, showing a weak decreasing trend over the observation time

Table 3. - Main parameters describing the variation of percent cover values (Maclvor and Lundholm, 2011) for the six studied species over the whole monitoring period (August 2015-May 2016). All values are average values ± standard deviation of the percent cover values recorded in each monitored module. The variation coefficient is given in brackets.

Species	**Percent cover on August 2015**	**Percent cover on May 2016**	**Maximum percent cover and month**	**Percent cover increase (May 2016-August 2015)**
S. minor	36 ± 17 (0.49)	73 ± 23 (0.32)	75 ± 21 (April) (0.28)	39 ± 16 (0.41)
F. ovina	10 ± 6 (0.55)	69 ± 22 (0.32)	69 ± 22 (May) (0.32)	59 ± 21 (0.35)
H. pilosella	25 ± 5 (0.22)	47 ± 19 (0.40)	47 ± 19 (May) (0.40)	22 ± 17 (0.77)
T. serpyllum	36 ± 9 (0.35)	38 ± 4 (0.09)	47 ± 13 (April) (0.28)	1 ± 1 (11)
A. millefolium	25 ± 17 (0.68)	38 ± 22 (0.58)	38 ± 22 (May) (0.58)	13 ± 21 (1.62)
A. alpinus	12 ± 4 (0.30)	19 ± 6 (0.33)	22 ± 12 (April) (0.54)	7 ± 8 (1.14)



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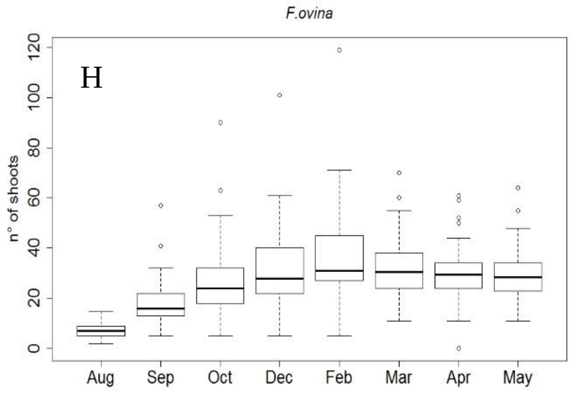
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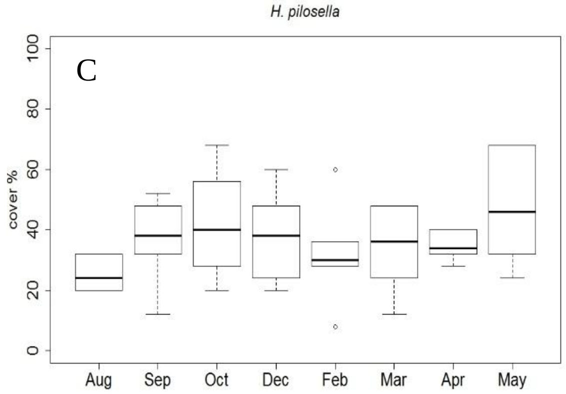
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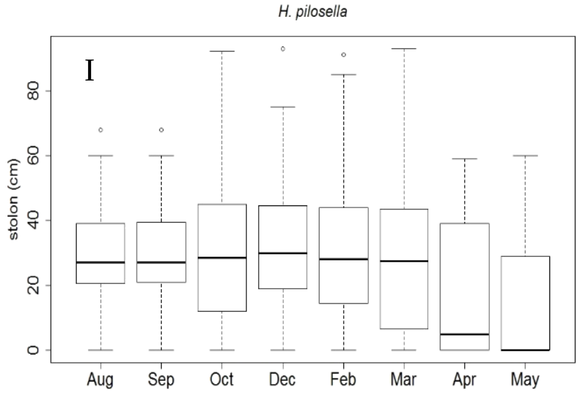
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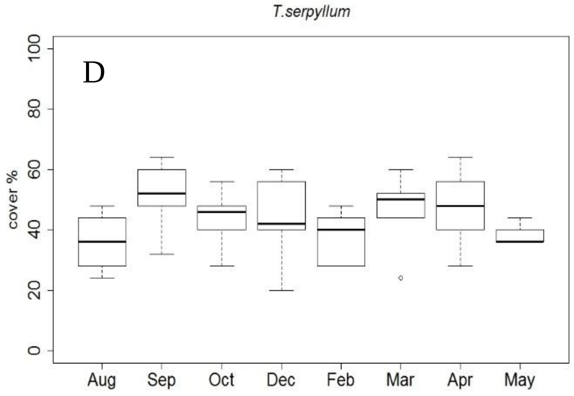
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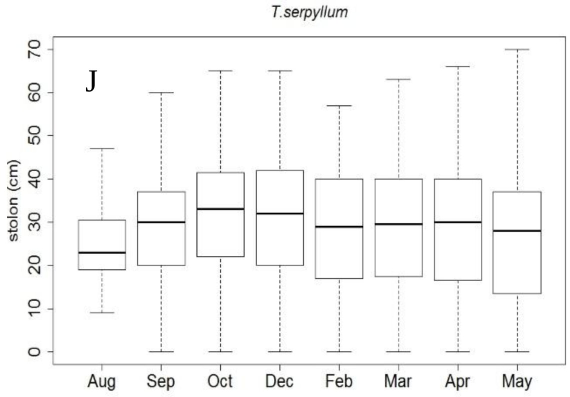
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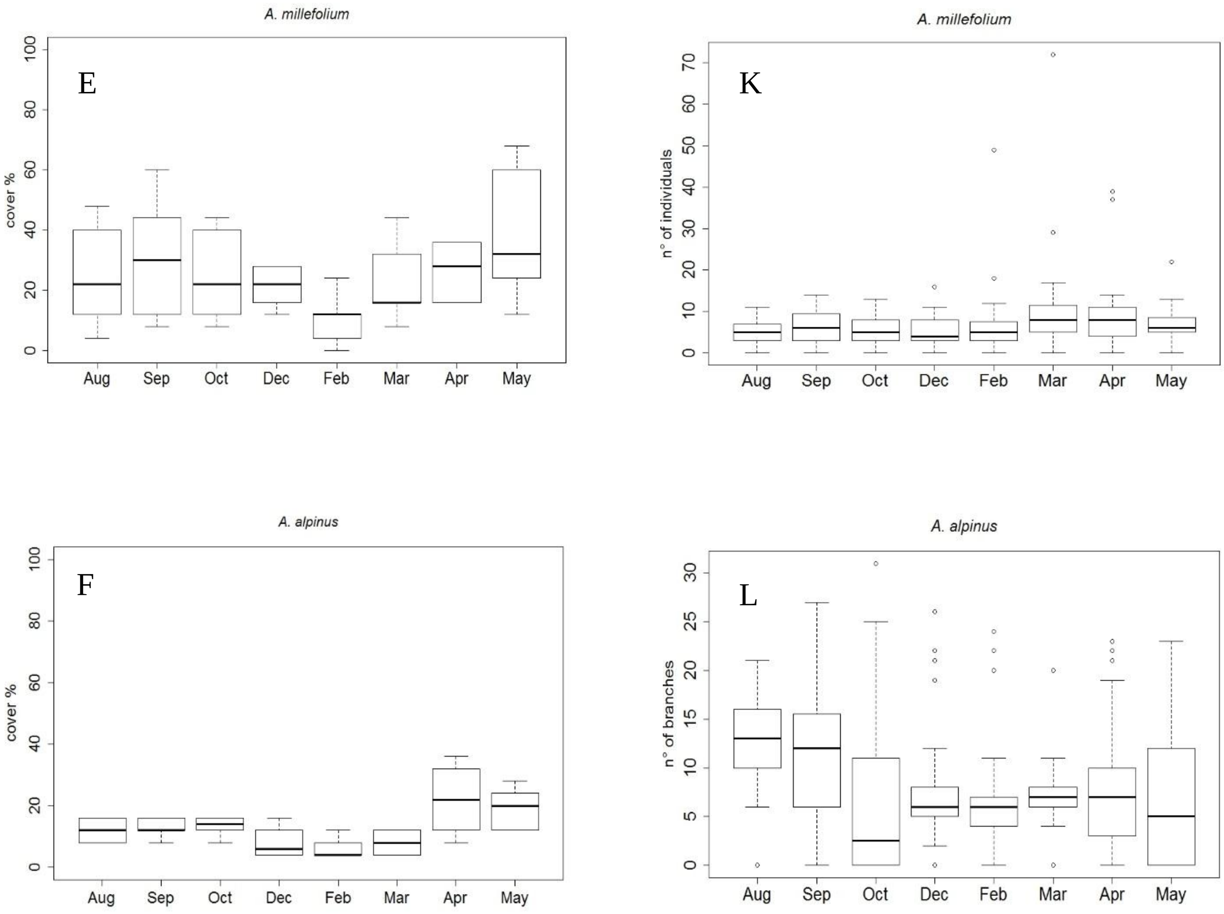

Figure 1. - In the graphs are reported the averages, 1st and 2nd quartile, sd and outliers. In the graphs from A to F are represented the percent cover values. From the graph G to the graph L are reported the values of different growth parameters.

The species behavior

For each of the studied species, Figure 1 shows the variation of the average percent cover, during the period August 2015 – May 2016, as well as the trends of growth of the morphological parameters monitored during the same time.

S. minor, the species with the highest final percent cover (73 % in May 2016 and 75 % in April 2016) showed a quick increase in the percent cover and in the area of the basal rosette during the first period of monitoring (August – October 2015), followed by a period of reduction in both parameters (October 2015 – February/March 2016), which again increased in the mid spring (April) 2016. The space occupied by this species varied depending on the seasons and months of the year, but is in any case of interest since even the minimum cover is above 50 %.

F. ovina reached a final percent cover very close to that of *S. minor*, but through a different way of growth, only moderately influenced by seasonality. Its occupation of the space increased progressively, though at different speeds, throughout the whole monitored period, except during the winter season. Its spring resprouting was precocious; the cover percentage and the number of shoots increased, respectively, from March and from February 2016.

A. alpinus showed only a limited ability to colonize the available space. The increase in percent cover during the overall monitored period is quite modest. The length of its branches strongly decreased in autumn-winter, recovering only a fraction of the initial length in the February-April period. The increase in percent cover in April 2016 is partly due to the production of new branches by the already existing individuals, partly to the increase in length of the monitored branches, and partly to new individuals originating from seeds of the previous year.

H. pilosella reached a moderate final percent cover characterized by slight seasonal oscillations. In contrast to the spring increase in cover, the length of the stolons showed a pronounced decrement during April and May. In fact, in this period of the year, a noticeable reorganization of the entire stolon system occurs: the old stolons die and new rosettes, producing in turn a new stolon system, are formed in May (mid spring) at the rooting points of the old stolons.

T. serpyllum has a growth pattern similar to that of *H. pilosella*. The elongation of the monitored stolons, after an initial noticeable growth, stopped and new stolons were formed (March and April) at the rooting points of the old stolons. With respect to *H. pilosella*, the disappearance of the old stolons is less evident and new biomass is produced earlier, at the start of spring, when temperatures are not so high.

A. millefolium shows a very dynamic pattern of space occupation, with an evident stationary period in winter. Vegetative propagation and sexual reproduction interacted reciprocally, concurring in the noticeable increment of percent cover from February to May 2016. The production of new shoots through vegetative propagation occurred very early, in February and March 2016, quickly recovering the number of units bearing reproductive structures (capitula). From March to May the percent cover increased mainly through the production of new individuals by sexual reproduction. Over one year, the production of new individuals balanced the death of the old ones.

Average cover values, however (Figure 2a), do not give enough information on the species with the best cover capacity overall the year. Average growth rate (Figure 2b), instead, can give more complete information on this important and interesting characteristic. *F. ovina* showed positive increment of coverage during the entire monitoring period. In the winter, the growth slows down, but the cover does not decrease significantly as for other studied species. This is an important aspect to consider, because during a period with a reduced plant coverage, many green roof properties could be reduced.

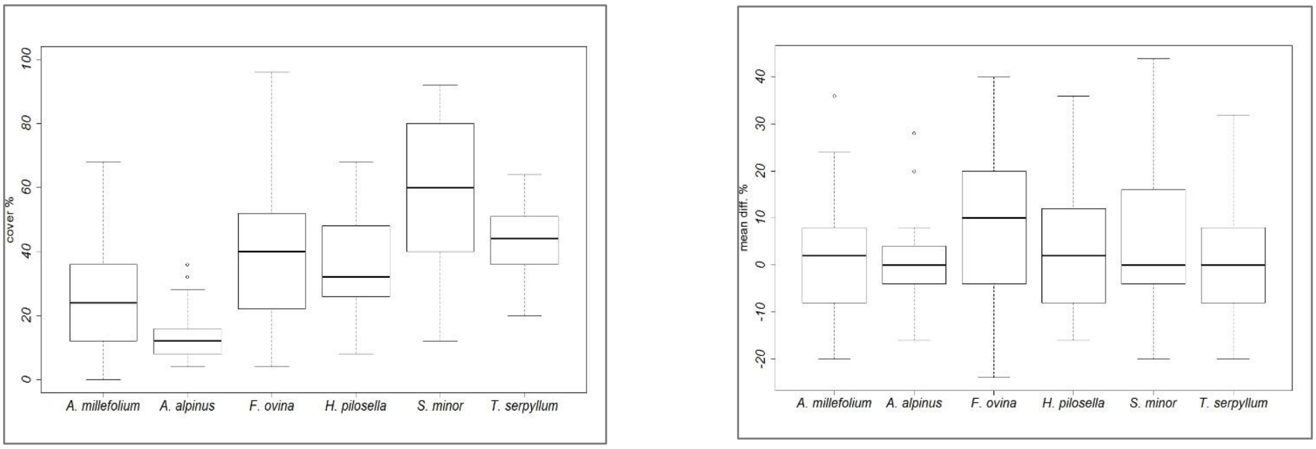

Figure 2a/2b - Average percent cover value of each species (left) and average growth rate (right) overall the monitoring period (August 2015 – May 2016).

Discussion

Even if the six studied species are very common and well known species of the Italian and European flora, the monitoring of their development over ten consecutive months allowed us to better understand their behavior in the colonization of space, the period of maximum/minimum cover, their persistence throughout the seasons and, on the whole, their pattern of growth, in particular on an extensive green roof, under the stratification, kind of substrate and management here described and adopted.

Among the species that produce a continuous cover, *F. ovina* is of particular interest because of its compact and dense tufts, persisting throughout the year. In spring and in autumn, as for all the microtherm grasses, when the mean monthly temperature ranges between 15 and 25 °C, *F. ovina* shows an active vegetative propagation (Verzotto, 2013), leading to a quick increment of shoots and of the size of the tufts, but even in winter the number of shoots do not decrease and the size of the tufts do not change significantly. Even if the species is highly tolerant to drought and to low fertility levels (Grime, 1988; Casler et al., 2003), a moderate fertilization and irrigation, as in the first period of our experiment, seems to improve its performances (Catalano et al., 2016).

The behaviour of *S. minor*, on the other hand, is quite different and of lesser interest due to the very pronounced seasonality of its cover. Both in our experience, and in that of other authors (Sydes and Grime, 1984; Grime, 1988), the rosettes of *S. minor* increased their size in spring and in autumn, reaching high percent cover values, but notably decreased in winter and, probably, also in summer (not reported here) because of the drought conditions typical of this period.

Finally, among the species with a compact structure, *A. alpinus* showed a much more marked seasonality, as well as a particular behaviour: the cover of the old individuals increased temporarily during a very limited spring period (Pignatti, 1982) and contributed little to the increment of the space occupied on the green roof modules. The effort for space occupation by this species mainly focuses on sexual reproduction (Bonnier, 1927; Brown, 1995) with an abundant seed production that will germinate during the next good season.

Another type of growth pattern, more dynamic, involving a periodical or continuous reorganization of the spatial distribution of the vegetative or of the reproductive structures characterizes the species that colonize the available space, forming a discontinuous cover with many empty patches. In terms of increase in cover percentage, the results are quite different for each species, but are in any case lower than those of *F. ovina* and *S. minor*. Species such as *H. pilosella* and *T. serpyllum* are subjected to a periodic re-arrangement of the positions where the new individuals, originated through vegetative propagation, developed and take nutrients. The contribution of sexual reproduction for the colonization of available space is not so significant, as the moderate increase in cover percentage of *H. pilosella* is due to the intense production of new stolons. Species such as A. *millefolium* instead, showed an important rearrangement in the spatial distribution of the new individuals originating from seeds and a modest modification of the percent cover of the vegetative propagation structures.

However, particular abilities in drought tolerance and/or in the use of water can significantly modify this general behaviour. *T. serpyllum*, for example, because of its creeping chamaephyte suffruticose habitus, can reduce transpiration (Schulze et al., 2005; Caneva et al., 2013) and more easily tolerate drought. Its use has thus been experimented on green roofs in many Mediterranean areas (Provenzano et al., 2010a; Vestrella et al., 2015). At the same time, as indicated by our data during the first period of monitoring, *T. serpyllum* can develop more quickly than *F. ovina*, if irrigated, and can sometimes become dominant (Vestrella et al., 2015).

These two different patterns of growth determine a different presence and coverage of the studied species over the year and, consequently, a different aptitude for their utilization in green roofs. It would be interesting to study how the compact species type could interact and persist when in competition with the spreading species type, on a green roof where they are contemporarily present. We hypothesize a decreasing ability of persistence, from *F. ovina*, *S. minor*, *A. millefolium*, *H. pilosella*, *T. serpyllum* to *A. alpinus*. Our findings are consistent with the observations of Pakeman et al. (2002) on natural grasslands, over a much longer period of years, where *H. pilosella* and *T. praecox* proved to be poorly persistent species in comparison with *S. minor* and *A. millefolium.*

Conclusions

The perennial species of the arid and semi-arid herbaceous plant communities represent a potentially interesting pool of species to use in the realization of plant covers for urban green roofs, as a valid alternative to the *Sedum* species. All the species under study survived and reproduced vegetatively and sexually during the experiment, so our study will hopefully increase the interest in the use of wild plant species on green roofs. However, the results obtained underlined the importance of a more precise knowledge of the behaviour of a group of different species, in order to select those more apt to carry out specific functions and ecosystem services.

Under the adopted management of irrigation and fertilization, only two of the tested species produced a good ground cover, and of these, only *F. ovina* can assure a well established cover throughout the year. In order to design green roofs with an appreciable specific biodiversity (Rosenzweig, 2016), species other than the dominant one should be taken into consideration, even if their cover capacity is less performing. A wide mixture of wild species on a green roof will increase the urban biodiversity and, consequently, improve the eco-system service potential (Cook-Patton *et al*., 2012; Blanusa *et al*., 2013). Among the tested species *A. millefolium* demonstrated a good colonization capacity through the abundant production of seeds and their efficient dispersal. By an appropriate calibration of irrigation and fertilization, better results could be obtained even from less performing species. We hypothesize that *H. pilosella* and *T. serpyllum*, for example, but in general all the species here considered, could positively respond to a little more availability of water and nutrients.

The proposal to use perennial species of arid and semi-arid herbaceous plant communities for green roofs and other urban Green Infrastructures can thus be positively evaluated, but will need further experiments in order to obtain the best possible results. Such experiments should define the most parsimonious water and nutrient management able to induce a satisfactory biomass production through minimal external inputs, thus exploiting the adaptations of these species to poor and limiting habitats (Heil and Diemont, 1983; Catalano *et al*., 2016).

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4. Final Discussion and Conclusions

The growing increasing public, industry and government interest in the diffusion of green roofs in the cities, thanks to their demonstrated and well-known benefits, encourages ~~the~~ researche~~s~~ to discover new advantages or to use new technologies in order to improve the already known benefits of these Green Infrastructures (GI), or to eliminate the disadvantages that hinder their spread.
This thesis is one of the many numerous studies that explore the benefits of these GI under different points of view (ecological, economic or human health); it pays particular attention to the role of the plant cover, as well as to the different advantages that green roofs can produce, depending on the different metabolism and physio-ecological adaptations of the species growing on them. As in the most recent papers (Wolf and Lundholm, 2008; Lundholm *et al.*2014; Lundholm *et al*. 2015), the results of this thesis demonstrate that the choice of the species is essential in order to obtain particular advantages from a green roof. Plant species with C3 or C4 metabolism, instead of the more commonly used CAM or CAM-facultative species, such as *Sedum* species, offer a wider range of environmental advantages and possibilities of mitigation in extreme conditions.
Results obtained from three years of indoor experimental tests and field observations, give some interesting insights on the real advantages (and limits) of the use of a group of native semi-xerophilus species on extensive green roofs built in Mediterranean or sub-Mediterranean areas~~,~~ under limiting growth conditions (water availability, summer and winter temperatures). The advantages concerning the summer cooling and winter insulation properties of green roofs derived from the use of plant species with C3 or C4 metabolism, instead of the more commonly used CAM or CAM-facultative species. Parameters directly or indirectly linked to (summer cooling and winter insulation properties) were measured in four different experiments.
As far as the summer cooling effects of green roofs are concerned, three different groups of species were explored:

- the CAM-facultative species *S. lydium* and *S. kamtschaticum*;
- the C3 species *B. erectus*, *L. corniculatus*, and *S. officinalis*;
- the C4 species *C. dactylon*.

The first group of species provides the lowest interesting cooling performances.
The diurnal water losses of *S. lydium* and *S. kamtschaticum* are about 0.3 g of water/g of plant material (value that increased to 0,4 g/g in the third experiment characterized by higher average temperatures) up to 11 % w/w of SMC.
Below these SMC values the diurnal water losses of the two *Sedum* species are very low, for example around 6 % w/w of SMC the diurnal water losses of *S. lydium* and *S. kamtschaticum* are about 0.1 g/g. This water saving behavior is due to the change of metabolism from C3 to CAM. This change occurs, as observed through the nocturnal increase of malic acid concentration, at between 11 % and 6 % w/w of SMC. At 12 % v/v of SMC, even a reduction of diurnal net assimilation rate has been observed.
A consequence of this behavior is a reduction of cooling performances too. As observed in the second experiment, up to 12 % v/v of SMC , the average deep temperatures recorded on the boxes planted with *S. lydium* and *S. kamtschaticum* were, on average, respectively 2 °C and 3 °C lower than the average the temperatures recorded below under the substrate without plants. Under 12 % v/v of SMC values, the differences between the average depth temperatures of *Sedum* boxes and the boxes with bare substrate decreased only by 1°C.
It must be noted that the great diffusion of green roofs vegetated with *Sedum* species, come from the ability of these species to accumulate water inside their leaves and stem, long time surviving under very strong drought conditions, with a very low water expenditure. This ability, deriving from a sparing use of water, makes these species much less interesting, if their cooling performances are considered. Paradoxically, in order to ensure good cooling performances by a green roof vegetated with CAM-facultative *Sedum* species, a high availability (upper to 11 % w/w or 12 % v/v of SMC) of water in the substrate should be maintained, through adequate irrigations.

The second group of species provides interesting cooling performances.
The C3 species tested (*B. erectus*, *L. corniculatus*, and *S. officinalis*) provide a greater cooling capacity than CAM-facultative species (*S. kamtschaticum* and *S. lydium*). This result strongly depends on their higher transpiration activities that, consequently, increase the evapotranspiration. The average diurnal water losses of *B. erectus* and *L. corniculatus* are on average of 0.6 g of water/g of plant material up to values of SMC of

10 % w/w, twice as much as the diurnal water loss of *Sedum* species, under the same substrate moisture. Moreover, transpiration of C3 species is active until about 6 % w/w of SMC, in fact, their average diurnal water losses are 0.5 g/g (instead of 0.1 g/g of *Sedum* species). This has a positive influence on the cooling effect because above 6 % v/v of SMC (when *Sedum* species already use CAM metabolism), the average depth temperatures recorded under *B. erectus* and *S. officinalis* covers, are 1 °C (0.5 °C for *L. corniculatus*) lower than the *Sedum* ones. These differences could be greater if the SMC of the boxes vegetated with C3 species was kept above 9 % v/v, avoiding water stress situations as well as the typical plant reactions, such as the rolling of the leaves by grasses. Rolled leaves, in fact, reduces transpiration, but reduces also the canopy coverage and, consequently, the shadow effect and his positive influence on the plant cooling capacity. Generally speaking, to ensure good cooling performances by a green roof vegetated with the above-mentioned C3 species, the SMC should be maintained slightly higher than 9 % v/v (or 10% w/w).

The third group provides very interesting cooling performances.

The C4 species tested, *C. dactylon,* instead, shows an intermediate behavior between the high water saving capacity of *Sedum* species and the good transpiration activity of C_3 plants.

In particular, the average diurnal water loss of *C. dactylon* is 0.5 g of water/g of plant material and does not change up to 5 % w/w of SMC, when the average diurnal water loss of *B. erectus* decreases to 0.4 g/g.

In addition, instead of C3 species, also the RWC values of *C. dactylon* leaves are kept high (more than 90 %) until to 5 % w/w of SMC, like the CAM-facultative species tested. Thus, C4 plants could provide the same or a better cooling effect, under water stress conditions, than C3 species.

In general, if a summer cooling effect is required, C3 and C4 species can provide more advantage than *Sedum* species, It is important to identify an appropriate water management, maintaining the SMC values, to which the desired cooling effect corresponds, identifying the minimum number of irrigation events and their volumes, necessary to guarantee a given cooling effect.

If only a plant cover is required, without any particular cooling effect, Sedum species are preferable.

As far as the winter insulating effects of green roofs are concerned, the performances of three species were explored.

B. erectus produces compact and thick turfs with a high quantity of biomass, during the growing season. These, even without vegetative activity, persist even in winter, maintaining the same structural characteristics that allow an effective mitigation of the low external temperatures.

For this reason, *B. erectus* shows the greatest ability to maintain, during the winter, the minimum temperatures below the growing layer warmer than the other species tested and the substrate without plants.

L. corniculatus and *C. dactylon* do not produce compact and thick turfs and, in addition, lose many leaves during the winter. *S. kamtschaticum* and *S. lydium*, because of the high water content of their leaves, suffer frequently freezing injuries, that can create necrosis and interruptions in the continuity of the cover.

Thermal contribution from the *B. erectus* coverage on the average minimum depth temperatures (-7 cm depth), ranges from a minimum of 0.6 °C to a maximum of 2 °C more than the average thermal contribution of the bare substrate. However, independently from the characteristics of their cover, all the studied species showed a greater insulation effect than the substrate without plants. On the other hand, during the daytime hours, when the sun heats the surface of green roofs after night, a consistent and thick plant cover reduces the solar heat gain and, consequently, the heat accumulated in the substrate before the next night (Lundholm *et al*. 2014). For this reason, in particular when the minimum night temperatures drop below zero, the substrate under a dense plant cover (like for *B. erectus*) needs more time to warm up, than the bare substrate.

In conclusion, in order to improve the thermal performances of the green roofs, C3 and C4 species can provide more interesting performances, than CAM-facultative *Sedum* species.

In summer, C3 species such as *B. erectus*, or *L. corniculatus*, or *S. officinalis* (or a mix of these species) can provide a significant cooling effect, if a regular, even if moderate irrigation, is available.

C4 species, such as *C. dactylon,* can represent, on the other hand, a very interesting solution to guarantee a discreet cooling effect, even in presence of low irrigation possibilities.

Mitigation of low winter temperatures requires a well-structured plant cover, thick and compact, such as that of *B. erectus* or other caespitose grasses. This point could be critical for the choice of species able to assure thermal advantages both in summer as in winter.

Many recent researches also dealt with the overall behavior of species not yet used for covering green roofs, but very widespread in local spontaneous floras (Benvenuti and Bacci, 2010; Benvenuti, 2014). The last experiment presented in this thesis deals with a preliminary analysis of growth, occupation of space and covering capacity of a group of perennial herbaceous species (*A. alpinus*, *A. millefolium*, *F. ovina*, *H.*

pilosella, *S. minor* and *T. serpyllum*) commonly found in semi-arid calcareous grasslands of the *Mesobromion*, widely spread in the basal belt of the Apennines.
Results of the experiment indicated that these species could represent a potentially interesting pool of *taxa*, to use in the green roofs, as a valid alternative to the *Sedum* species. All species studied~~,~~ survived and reproduced on the roof~~,~~ but the results underlined important differences in growth behavior between the species. Under the low management of irrigation and fertilization adopted during the experiment, only two species provided good coverage, and of these, only *F. ovina* assured a well-established cover throughout the year. *F. ovina* showed the capacity to preserve, even in winter, a dense coverage. However, *F. ovina* has a slow spreading capacity, unlike *A. alpinus* and *A. millefolium*. *A. millefolium*, in particular, demonstrated a good colonization ability through the abundant production of seeds and their efficient dispersal, in addition to a remarkable production of hypogeal stolons. *A. alpinus* and *A. millefolium* can guarantee a quick colonization of empty spaces due~~,~~ to the death of some individuals, with higher coverage (such as *F. ovina*). *A. alpinus* and *A. millefolium* can thus perform a "filling" function. In addition, *H. pilosella* and *T. serpyllum*, stoloniferous and creeping species, under low irrigation and fertilization management did not show significant growth capacities. Probably, through an appropriate calibration of irrigation and fertilization at least during the first two years after planting, better results could be obtained even from less performing species.

To conclude, the use of C3 perennial native species of arid and semi-arid herbaceous communities, instead of the CAM-facultative *Sedum* species, can be positively evaluated, but it needs careful species selection~~,~~ to define the best plant composition, depending on the climatic context and the frequency of irrigation and fertilization events.

A **mixture of wild species could increase the urban biodiversity but also could improve the eco-system services of green roofs**. In fact, a mix of species with different growth behaviors may be more efficient than a monospecific roof~~,~~ because **high biodiversity increases the resilience of the green roof necessary to overcome the numerous stress events during the year** (Cook-Patton and Bauerle, 2012)**.**

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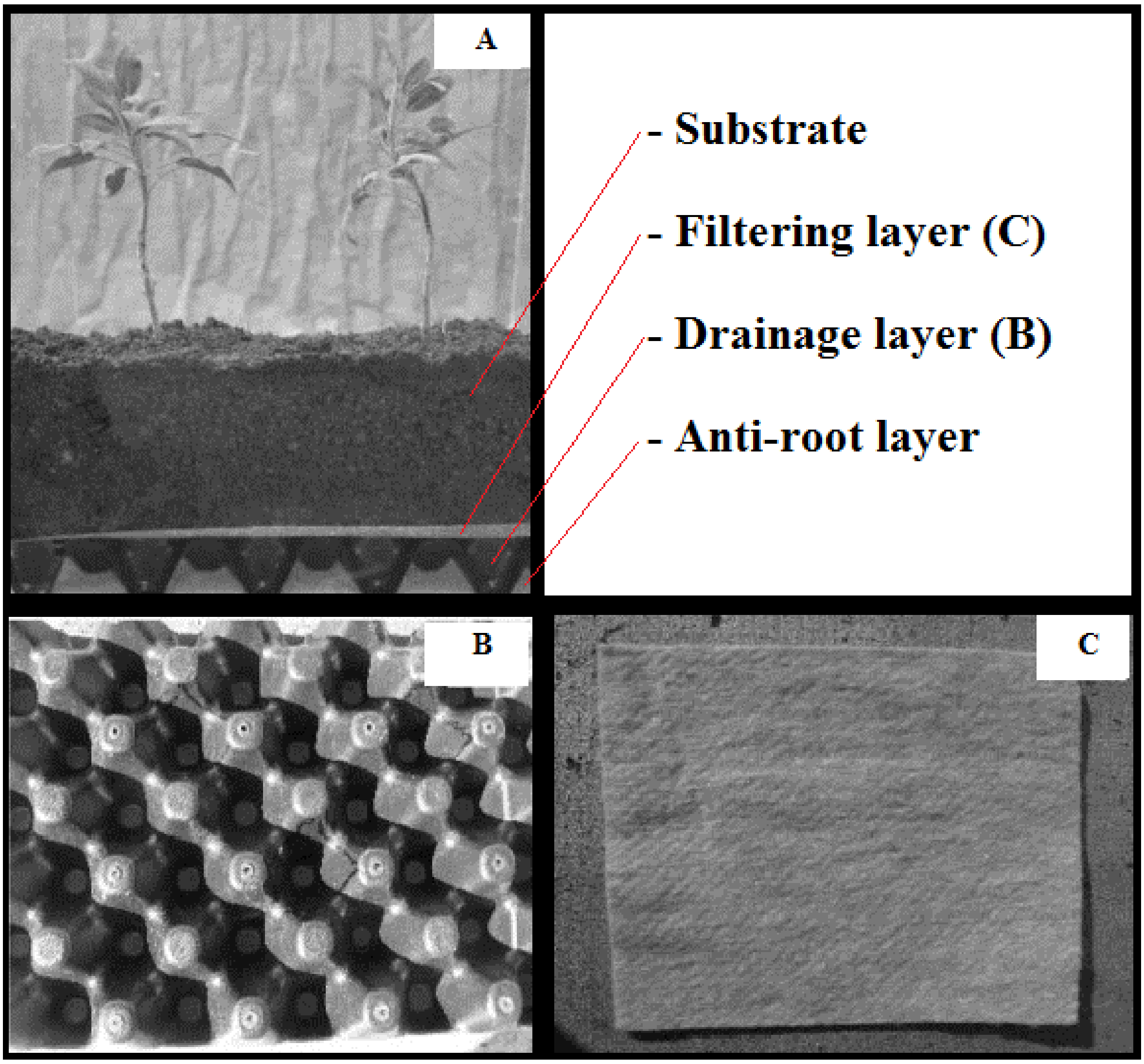
ANNEXES

Annex 1

NOMENCLATURE

Main variables	Abbreviation	Unit
Net CO_2 assimilation rates	A	μmol CO_2 m^{-2} s^{-1}
Extensive Green Roof	EGR	
Green Infrastructure	GI	
Intensive Green Roof	IGR	
Leaf Area Index	LAI	Unitless (or m^2 leaf area m^{-2} ground area)
Leaf stomatal conductance to H_2O	g_s	mol H_2O m^{-2} s^{-1}
Least Significant Difference	LSD	
Relative Water Content	RWC	%
Substrate Moisture Content	SMC	%
Transmission Loss	TL	dB
Transpiration rate	E	mmol H_2O m^{-2} s^{-1}
Urban Heat Island effect	UHI	

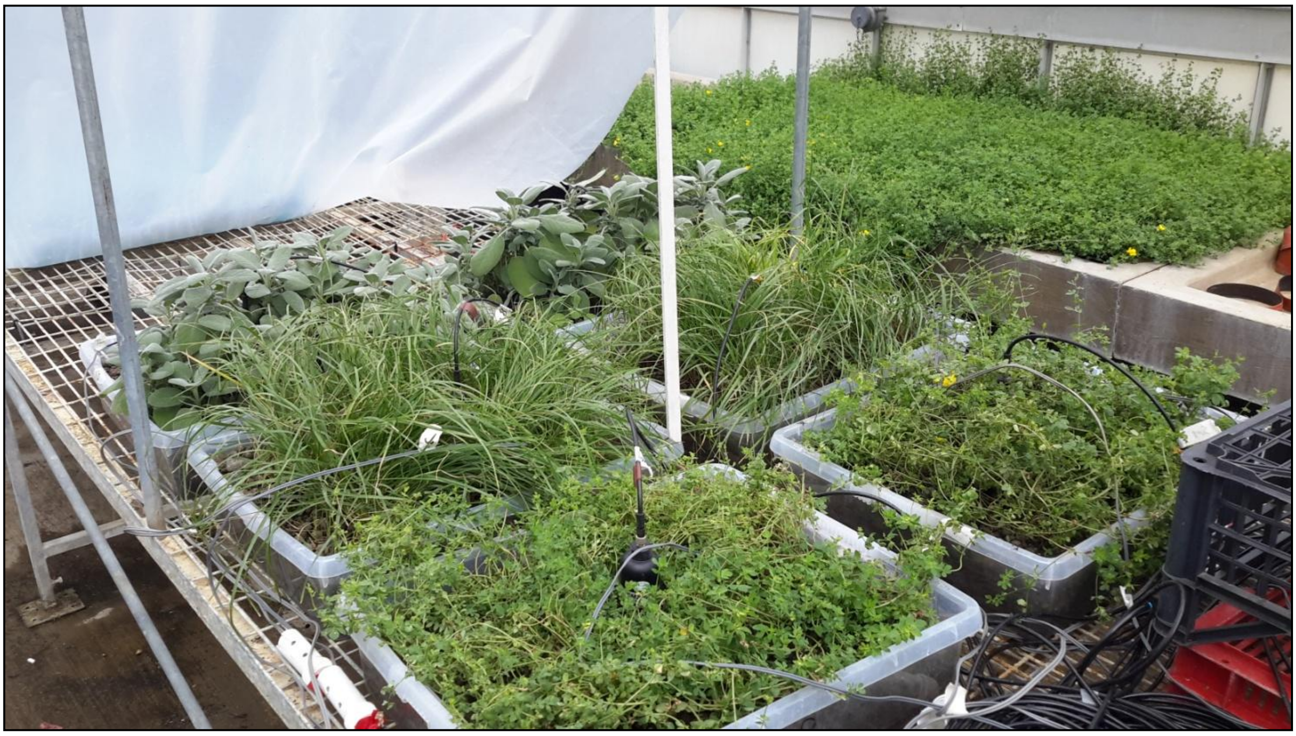


Photograph (A) of the stratification used in the 1st, 3rd and 4th experiment. The stratification used included drainage plastic profiled panels with holes to favor water transfer between different layers (B) and filtering layer (C) and anti-root layer (Savi *et al*., 2013).

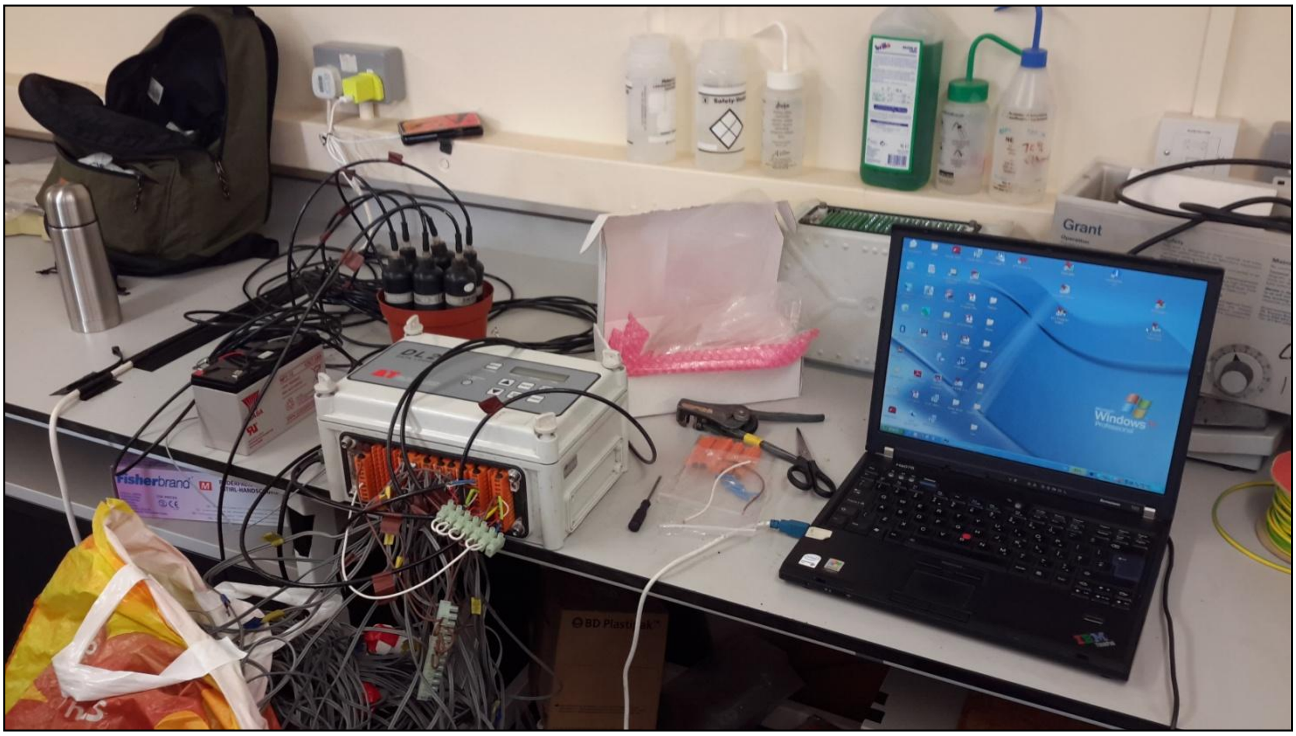
Annex 3

The boxes planted with the four species during the 1st and 3rd experiment. Both experiments were carried out in the glasshouse of the DipSA Department (University of Bologna, Italy).

Annex 4



Six of the twelve boxes prepared for the 2nd experiment conducted in the glasshouses of School of Agriculture, Policy and Development, University of Reading (UK).



The photograph shows the calibration phase of SM200 soil moisture probes (Delta-T devices Ltd., Cambridge, UK) and thermistors (type T fine PTFE insulated twin twisted wires) connected to the DL2e data logger (Delta-T Devices Ltd, Cambridge, UK).

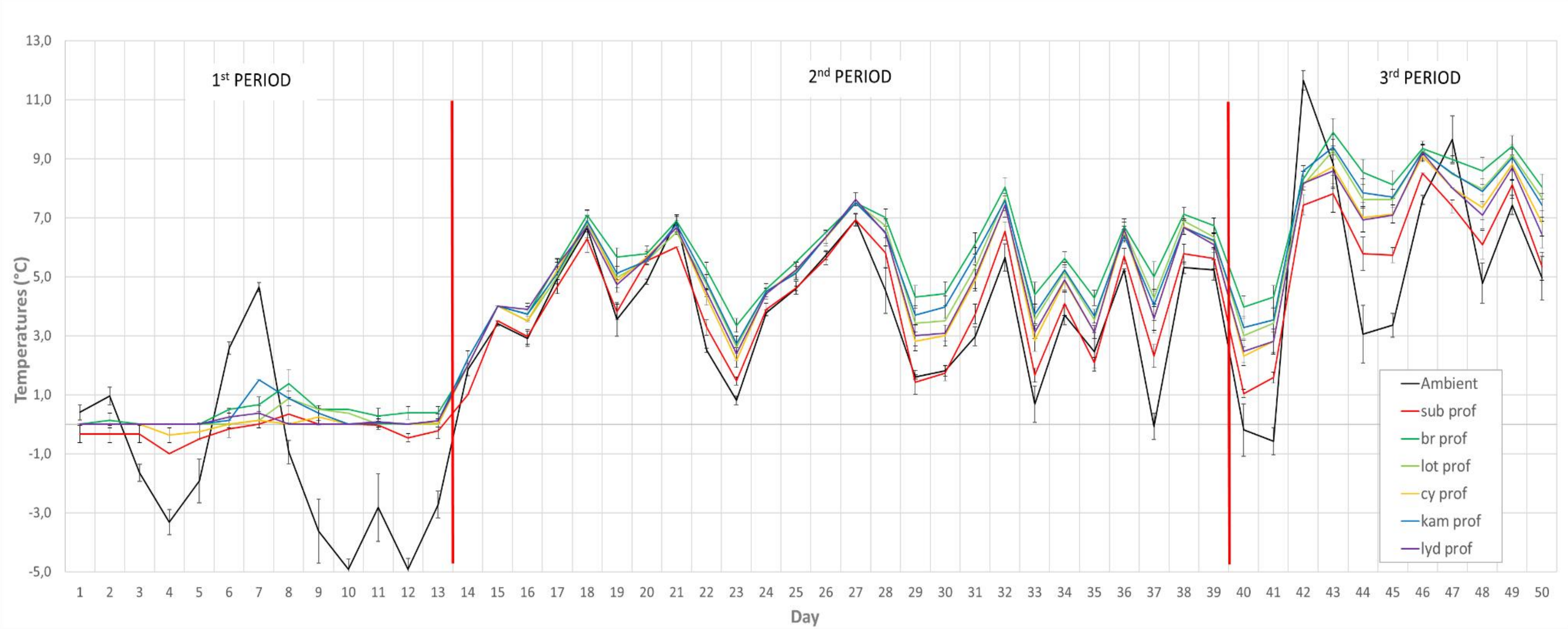


Figure 2.5.2 – Average daily minimum temperatures (± sd) recorded at 7 cm depth in the substrate of each boxes and in the atmosphere (Ambient), by a meteorological station close to the place of the experiment. sub prof = Bare substrate, br prof = *B. erectus*, lot prof = *L. corniculatus*, cy prof = *C. dactylon*, lyd prof = *S. lydium*, kam prof = *S. kamtschaticum*.

Annex 6

Table 2.5.1 – Daily average minimum temperature at 7 cm depth (DMT), on the surface (SMT) and their differences (DMT-SMT) of each box, during the experiment.

Day	**Bare substrate**			***B. erectus***			***L. corniculatus***			***C. dactylon***			***S. kamtschaticum***			***S. lydium***		
	DMT (°C)	**SMT (°C)**	**diff (°C)**	**DMT (°C)**	**SMT (°C)**	**diff (°C)**	**DMT (°C)**	**SMT (°C)**	**diff (°C)**	**DMT (°C)**	**SMT (°C)**	**diff (°C)**	**DMT (°C)**	**SMT (°C)**	**diff (°C)**	**DMT (°C)**	**SMT (°C)**	**diff (°C)**
1	-0.3	-0.6	0.3	0.0	-0.1	0.1	0.0	0.0	0.0	0.0	-0.3	0.3	0.0	-0.4	0.4	0.0	0.0	0.0
2	-0.3	-0.1	-0.2	0.1	0.4	-0.3	0.0	0.0	0.0	0.0	0.4	-0.4	0.0	0.4	-0.4	0.0	0.0	0.0
3	-0.3	-2.0	1.7	0.0	-0.8	0.8	0.0	-0.5	0.5	0.0	-1.4	1.4	0.0	-1.0	1.0	0.0	-0.5	0.5
4	-1.0	-2.3	1.3	0.0	-1.0	1.0	0.0	-0.9	0.9	-0.4	-1.6	1.3	0.0	-1.6	1.6	0.0	-1.0	1.0
5	-0.5	-1.3	0.8	0.0	-0.5	0.5	0.0	-0.4	0.4	-0.3	-0.5	0.3	0.0	-0.8	0.8	0.0	-0.8	0.8
6	-0.2	0.8	-0.9	0.5	1.0	-0.5	0.0	0.5	-0.5	0.0	1.1	-1.1	0.1	1.6	-1.5	0.3	0.6	-0.4
7	0.0	0.9	-0.9	0.7	2.1	-1.5	0.1	0.8	-0.6	0.1	1.5	-1.4	1.5	2.9	-1.4	0.4	1.3	-0.9
8	0.3	-0.1	0.5	1.4	-0.5	1.9	0.9	0.0	0.9	0.0	-0.6	0.6	0.9	-0.6	1.5	0.0	-0.3	0.3
9	0.0	-1.3	1.3	0.5	-1.0	1.5	0.5	-0.4	0.9	0.3	-1.6	1.9	0.4	-1.8	2.1	0.0	-1.0	1.0
10	0.0	-1.5	1.5	0.5	-1.0	1.5	0.4	-0.5	0.9	0.0	-1.5	1.5	0.0	-1.9	1.9	0.0	-1.0	1.0
11	0.0	-0.3	0.3	0.3	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-0.6	0.7	0.1	-0.4	0.5
12	-0.5	-1.7	1.2	0.4	-0.8	1.2	0.0	-0.3	0.3	0.0	-1.4	1.4	0.0	-1.6	1.6	0.0	-1.0	1.0
13	-0.2	-1.2	1.0	0.4	-0.5	0.9	0.0	0.0	0.0	0.0	-0.9	0.9	0.1	-1.0	1.1	0.1	-0.6	0.7
14	1.0	1.3	-0.3	2.0	2.0	0.0	2.0	2.0	0.0	2.0	2.1	-0.1	2.2	2.2	0.1	2.0	2.0	0.0
15	3.5	3.6	-0.1	4.0	4.0	0.0	4.0	4.0	0.0	4.0	4.0	0.0	4.0	4.0	0.0	4.0	4.0	0.0
16	3.0	3.5	-0.6	3.7	3.6	0.2	3.5	3.6	-0.1	3.5	3.8	-0.3	3.7	3.8	-0.1	3.9	3.8	0.1
17	4.7	4.8	-0.2	5.4	5.0	0.4	5.0	5.0	0.0	5.2	5.2	0.0	5.1	5.0	0.1	5.4	5.0	0.4
18	6.3	4.8	1.4	7.1	6.3	0.8	6.8	6.1	0.7	6.9	5.7	1.2	6.9	5.8	1.1	6.7	6.0	0.7
19	3.8	2.6	1.2	5.7	4.4	1.2	5.0	4.0	0.9	4.8	3.5	1.4	5.1	3.6	1.5	4.7	3.9	0.8
20	5.5	5.0	0.5	5.8	5.1	0.7	5.5	5.5	0.0	5.7	5.5	0.2	5.5	5.0	0.5	5.6	5.5	0.1
21	6.0	5.8	0.2	6.9	6.4	0.5	6.5	6.3	0.2	6.7	6.3	0.4	6.8	6.3	0.5	6.7	6.3	0.4
22	3.3	2.3	1.0	5.2	4.0	1.2	4.7	3.7	1.1	4.3	3.3	1.0	4.8	3.3	1.5	4.5	3.6	0.8
23	1.5	0.4	1.1	3.3	2.0	1.3	2.6	1.6	1.0	2.2	1.1	1.0	2.7	1.1	1.6	2.4	1.1	1.3
24	3.9	3.5	0.4	4.6	4.0	0.6	4.5	4.0	0.5	4.5	4.0	0.5	4.5	3.7	0.8	4.4	4.0	0.4
25	4.6	4.5	0.1	5.5	5.0	0.5	5.2	5.0	0.2	5.2	5.0	0.3	5.1	4.6	0.5	5.2	5.0	0.2
26	5.6	5.5	0.2	6.5	6.0	0.5	6.3	6.0	0.3	6.3	6.0	0.3	6.3	5.9	0.5	6.3	6.0	0.3
27	6.9	6.7	0.3	7.5	7.4	0.1	7.5	7.3	0.2	7.5	7.2	0.3	7.5	7.0	0.5	7.6	7.2	0.4
28	5.8	4.1	1.7	7.0	5.7	1.3	6.7	5.6	1.1	6.5	5.0	1.5	6.5	4.8	1.7	6.5	5.3	1.2
29	1.4	-1.1	2.5	4.3	2.4	1.9	3.4	1.7	1.7	2.8	1.0	1.8	3.7	1.2	2.5	3.0	1.7	1.3
30	1.7	-0.8	2.5	4.4	2.6	1.8	3.5	1.8	1.7	3.0	1.0	2.0	4.0	1.4	2.5	3.1	1.5	1.5
31	3.7	2.0	1.7	6.1	4.7	1.4	5.3	4.1	1.2	4.9	3.5	1.3	5.7	4.0	1.7	5.0	4.1	0.9
32	6.5	4.6	1.9	8.0	6.6	1.4	7.7	6.5	1.2	7.4	5.8	1.7	7.6	5.7	1.9	7.4	6.0	1.5
33	1.7	0.3	1.3	4.4	2.2	2.2	3.5	1.8	1.7	2.8	1.1	1.7	3.7	1.2	2.6	3.1	1.7	1.4
34	4.1	2.7	1.4	5.6	4.2	1.5	5.2	3.9	1.2	4.8	3.5	1.3	5.2	3.5	1.7	4.9	3.8	1.1
35	2.1	0.6	1.5	4.3	2.9	1.4	3.5	2.3	1.2	3.1	1.9	1.2	3.7	2.2	1.5	3.1	2.2	0.9
36	5.7	5.1	0.6	6.7	5.7	1.0	6.6	6.1	0.5	6.5	5.7	0.7	6.4	5.5	0.9	6.6	5.9	0.7
37	2.3	-0.3	2.6	5.0	2.5	2.5	4.3	2.3	1.9	3.6	1.4	2.2	4.0	1.0	3.0	3.6	1.6	2.0
38	5.8	4.7	1.0	7.1	6.0	1.1	6.9	6.2	0.7	6.7	5.6	1.1	6.7	5.2	1.4	6.7	5.7	0.9
39	5.6	4.0	1.6	6.7	5.4	1.3	6.3	5.4	0.9	6.2	4.8	1.3	6.2	4.7	1.5	6.1	5.0	1.1
40	1.0	-0.7	1.8	4.0	1.5	2.4	3.0	1.2	1.8	2.3	0.4	1.9	3.3	0.3	3.0	2.5	1.0	1.5
41	1.6	-0.3	1.9	4.3	1.7	2.6	3.4	1.5	2.0	2.8	0.6	2.2	3.5	0.6	2.9	2.8	1.3	1.5
42	7.4	9.3	-1.9	8.3	9.7	-1.3	8.1	8.3	-0.2	8.2	9.5	-1.3	8.6	9.6	-1.0	8.2	8.7	-0.5
43	7.8	6.3	1.5	9.9	8.1	1.8	9.3	7.3	1.9	8.7	7.0	1.7	9.4	7.8	1.6	8.6	7.5	1.1
44	5.8	3.2	2.6	8.5	6.1	2.5	7.6	5.7	2.0	7.0	4.8	2.2	7.8	4.9	3.0	6.9	5.3	1.6
45	5.7	4.4	1.3	8.1	6.5	1.6	7.6	6.3	1.3	7.1	5.7	1.4	7.7	6.0	1.7	7.1	6.0	1.1
46	8.5	8.0	0.5	9.3	8.4	1.0	9.3	8.9	0.3	9.1	8.5	0.5	9.2	8.4	0.8	9.2	8.7	0.5
47	7.4	7.6	-0.2	9.0	8.3	0.7	8.5	7.8	0.6	8.0	8.1	-0.1	8.5	8.3	0.2	8.0	7.8	0.2
48	6.1	3.5	2.5	8.6	6.3	2.3	8.0	6.0	2.0	7.3	5.3	2.0	7.9	5.6	2.3	7.1	5.6	1.5
49	8.1	6.5	1.6	9.4	7.5	2.0	9.1	8.0	1.2	8.8	7.1	1.7	9.0	7.3	1.8	8.7	7.7	1.0
50	5.3	3.2	2.1	8.0	5.5	2.6	7.7	5.7	2.0	6.8	4.4	2.4	7.4	4.9	2.5	6.4	4.9	1.5

Annex 7

The experimental green roof planted with native species built in the Agricultural Sciences School of the Bologna University (Italy). The stratification adopted has a total depth of about 18 cm, where the upper part is organized in quadrate modules of 54 x 54 x 9 cm depth, made of regenerated plastic, interlocking each other and filled with pumice grains (Ø 3-6 mm) and with VULCAFLOR® up to the brim.

A. alpinus and *A. millefolium* individuals, originated by seed dissemination, invaded the modules of *H. pilosella*.