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Morphological and architectural traits of fine roots in *Salvia nemorosa* and *Sanguisorba minor* across four Central European botanical gardens

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Abstract

Despite recently increased scientific efforts, subterranean plant organs and their relations to weather and aboveground plant development are still severely understudied. To contribute to a more thorough understanding of absorptive roots' relationships with weather variables and phenological progression, two species observed as part of an international project monitoring phenology are scrutinised in the present thesis. A four-year observation of the aboveground phenology of *Salvia nemorosa* and *Sanguisorba minor* in four Central European botanical gardens was combined with publicly available weather data and destructively sampled absorptive root measurements. From the resulting data set, key statistical measures for the root parameters were calculated and their correlations with weather and phenology were assessed. Morphological and architectural root traits were found to be highly variable, with only average diameters in *S. minor* and root branching density in both species appearing relatively stable across the locations. Root traits correlated weakly but extensively with weather data. High temperatures correlated negatively with average diameters and specific root area but positively with root dry matter content and root tissue density. Patchy precipitation correlated negatively with average diameters. These findings indicate a previously unobserved strategy of increasing the root-soil interactive surface for improved substance uptake at low carbon cost during specific abiotic stresses on the plant. Strong correlations found between root traits and phenology, particularly reproductive phenophases, suggest that stark branching and large soil-root interfaces, which are associated with resource acquisitive strategies, coincide with fast phenological development. Large diameters and high root tissue densities, on the other hand, co-occurred with a slower, more drawn-out phenological progression. These results show that, corresponding to the current understanding of the root economics space, root traits associated with fast and slow strategies do indeed correlate with according phenological progressions. Overall, this thesis' findings reiterate the crucial importance of plants' roots for their functioning, adaptability to their environments, and the coordinated regulation of above- and belowground development.

Zusammenfassung

Trotz verstärkter Forschung in den letzten Jahren ist nach wie vor wenig über unterirdische Pflanzenteile und deren Wechselbeziehungen mit Wettereinflüssen und der Entwicklung oberirdischer Organe bekannt. Um zu einem besseren Verständnis des Zusammenhangs von aufnahmefähigen, feinen Wurzeln mit Wetter und Phänologie beizutragen, beschäftigt sich die vorliegende Arbeit mit zwei Arten, die im Zuge eines internationalen Projekts zur phänologischen Beobachtung krautiger Pflanzen untersucht wurden. Ein vier Jahre dauerndes Monitoring der Phänologie von *Salvia nemorosa* und *Sanguisorba minor* in vier mitteleuropäischen botanischen Gärten wurde mit öffentlich verfügbaren Wetter- und destruktiv gewonnenen Wurzeldaten kombiniert, um daraus Wurzelmerkmale und ihre Korrelationen mit Wetter- und phänologischen Parametern zu berechnen. Die morphologischen und architektonischen Merkmale erwiesen sich als hochvariabel, lediglich die durchschnittlichen Durchmesser bei *S. minor* und die Dichte der Wurzelverzweigungen bei beiden Arten waren über die Standorte hinweg stabil. Korrelationen der Wurzelmerkmale mit den Wetterparametern waren zwar schwach, aber umfangreich. Hohe Temperaturen korrelierten negativ mit durchschnittlichen Durchmessern und der spezifischen Wurzeloberfläche, während sie mit Trockenmasse und Gewebedichte eine positive Beziehung aufwiesen. Unregelmäßiger Niederschlag zeigte negative Korrelationen mit durchschnittlichen Durchmessern. Diese Ergebnisse weisen auf eine bislang unbekannte Strategie hin, mit der Pflanzen unter spezifischen Stressbedingungen und bei geringer Verfügbarkeit von Kohlenstoff ihre Stoffaufnahme über die Wurzeln verbessern könnten. Zwischen Wurzelmerkmalen und insbesondere den reproduktiven Phänophasen wurden starke Korrelationen gefunden, die auf ein gemeinsames Auftreten schneller Sprossentwicklung mit starker Wurzelverzweigung und großer -oberfläche schließen lassen. Diese Wurzelmerkmale sind allgemein mit schnellen Stoffwechselstrategien und kurzer Lebensdauer assoziiert. Große Durchmesser und hohe Wurzelgewebedichten hingegen korrelierten mit einer langsameren phänologischen Entwicklung. Diesen Erkenntnissen zufolge treten Merkmale, die mit einem schnellen Stoffumsatz in den Wurzeln einhergehen, auch gemeinsam mit einer schnellen oberirdischen Entwicklung auf, was im Einklang mit dem bisherigen Verständnis des Stoffwechselhaushalts von Wurzeln steht. Die Ergebnisse dieser Arbeit unterstreichen die Bedeutung der Wurzeln für die Funktion und Anpassungsfähigkeit von Pflanzen und der Koordination ihrer über- und unterirdischen Entwicklung.

1 Introduction

1.1 Plant roots and noteworthy traits

Root research has seen a recent surge of interest, in what B. Wang *et al.* (2023: 2880) have termed the "belowground trait data revolution". During the last several years, the scientific community has tackled many of the extant research gaps in the understanding of roots' anatomical, morphological, architectural, and chemical traits and their involvement in plant functioning (Freschet, Roumet *et al.* 2021: 1125-1133). In the decades prior, a tendency to focus on aboveground plant organs had resulted in these traits and functions, as well as roots' overall contribution to the biosphere, being largely overlooked (Norby & Iversen 2017: 6; B. Wang *et al.* 2023: 2871; Weemstra, Kuyper *et al.* 2023: 2). As organs of carbon allocation, water and nutrient uptake, phytohormone production, substance exudation, symbiosis, and numerous other physiological processes, fine, acquisitive roots constitute highly metabolically active plant organs. They alone are estimated to account for 22% of the global terrestrial net primary production (McCormack *et al.* 2015: 510). Together with older, coarser roots that fulfil structural, conductive, and storage functions in plants (Klimešová *et al.* 2018: 2118; Zhou *et al.* 2022: 431-432), such fine roots constitute a large proportion of plant biomass and play a vital role in global carbon storage (Radville *et al.* 2016: 3624-3625). Through their interactions with aboveground plant organs, roots also contribute substantially to overall ecosystem functioning. While they compete with shoots for the photosynthetically fixed carbon required to build their biomass, carbon investments in belowground organs can facilitate nutrient acquisition via the roots, which in turn benefits the shoots by enabling faster carbon assimilation (Weemstra, Kuyper *et al.* 2023: 8). Ultimately, plant performance relies on the interaction of interdependent organs above and below the soil surface. Depending on the environmental conditions they are faced with, plants must strike a delicate balance between investment in roots and shoots to optimise their growth, nutrient uptake, and photosynthesis (S. Wang *et al.* 2021: 82).

Despite this general awareness of plant roots' relevance, and although the body of empirical knowledge on the subject continues to grow, our understanding of roots' connections with aboveground plant traits, soil conditions, or surrounding ecological processes still leaves much to be desired. Whole-plant investigations on roots' trait-functioning relationships and their importance for overall plant performance, which determine their contribution to ecosystem functioning, are still sorely lacking (Freschet, Roumet *et al.* 2021: 1123, 1134; McCormack *et al.* 2017: 27). This is owed partly to

challenges that inevitably arise with observations of fragile tissue buried in an opaque substrate (Freschet, Pagès *et al.* 2021: 974-975; Klimešová *et al.* 2018: 2120; Norby & Iversen 2017: 5). Because careful and labour-intensive preparations are required for investigations of roots, corresponding research has been conducted more intensively on herbaceous plants. For these, collecting root data is more easily achievable than for woody species that are larger, longer-lived, and whose roots are particularly challenging to observe or harvest (McCormack *et al.* 2017: 27-28). Practical problems thus pose considerable limitations on the understanding of plant roots.

Challenges in the field also arise even with issues as fundamental as consistent descriptions and accurate measurements of root traits. Roots lack the discreteness other, more easily measurable plant organs possess, rendering their rather continuous properties difficult to quantify (Kong *et al.* 2014: 867-868; McCormack *et al.* 2015: 506). To mitigate the resulting potential for ambiguity in the description of roots, Freschet, Pagès, *et al.* (2021) propose a unified corpus of denominations and definitions for root traits that provides a comprehensive and universal system of trait conceptions, reduces semantic heterogeneity, and facilitates the collection of comparable results across the research field. This thesis follows their suggested nomenclature to contribute as best as possible to a standardised approach to the investigation of roots.

1.1.1 Morphometric classification

Root classification systems are typically based on either ontogenetic development, diameter, function, or order of roots. For the latter, following the branching hierarchy of roots from the distal tips to the proximal roots in a morphometric approach (also termed stream-order, Strahler, or centripetal approach) produces a useful classification that is translatable to different individuals, species, and growth types with potentially varying root system structures (Freschet, Pagès *et al.* 2021: 981-984). In contrast to other approaches, this system, originally described by Fitter (1982: 313), permits an unambiguous classification also of roots that may be incomplete, damaged, or in other ways deviant from a lab-grown ideal root. This renders it best suitable for research endeavours concerning field-grown root systems, especially ones that can only be partially harvested. Its use has strongly been advocated for to ensure comparability across root studies (McCormack *et al.* 2015: 514). For a morphometric classification, in a system illustrated in Figure 1, the distal root tips are assigned a root order of 1 and followed inwards to the root system’s centre, with the order values increasing by 1 every time two roots of the same order merge. As roots age and gradually develop more lateral

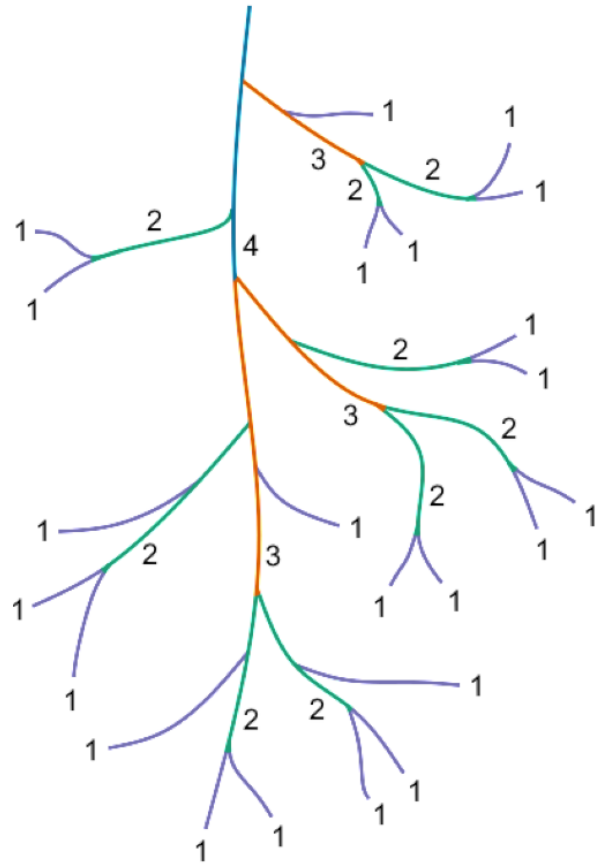

Figure 1 Schematic illustration of morphometric root classification. Root sections are coloured according to order: violet = 1, green = 2, orange = 3, blue = 4.

roots, the same section of a root may therefore successively traverse several morphometrically defined root orders across its ontogeny (Fitter 1982: 313-314). Such morphometric classification approaches are particularly useful when combined with computer-assisted image processing, which enables a time- and cost-efficient collection of trait data for large amounts of root material (Christodolou *et al.* 2020: 385). Studies using this morphometric system have found first-, second-, and third-order roots to show consistent mycorrhizal colonisation and anatomical adaptations for substance uptake (McCormack *et al.* 2015: 507). In dicotyledons, fourth- and higher order roots have been identified to possess cork layers or thickened exodermis cell walls that impede their capability for substance uptake. Thus, while there are considerable structural differences within the first three root orders (B. Wang *et al.* 2023: 2872; Zhou *et al.* 2022: 425-426), they can be functionally summarised as the absorptive fraction of a plant's root system, as opposed to the roots of order 4 and above, which fulfil transport and storage functions (Klimešová *et al.* 2018: 2116).

1.1.2 Commonly surveyed traits

Frequently investigated morphological and architectural root traits include diameter, length, tissue density, dry matter content, and branching density (Iversen *et al.* 2017: 18-19). These are often applied to subsections of root systems, such as particular root orders, to provide detailed insights into specific root system components. If traits are not measured for entire root systems, their values must be adjusted to ensure independence from the quantity of sampled material, and to enhance comparability across research efforts. This is particularly relevant in field surveys where root systems are often only partially recovered (Freschet, Pagès *et al.* 2021: 979, 981). One such approach is to relate values of root mass, length, surface area, and other parameters to the overall mass or volume of the collected root material to arrive at trait values

per unit of mass or volume, thus preventing distortions of data due to dissimilar amounts of root material harvested in different surveys. This frequently applied procedure also determines most of the parameters detailed below. Morphological characteristics, such as the first five traits listed, provide insights into root construction costs, root life span patterns, and nutrient uptake strategies (McCormack *et al.* 2017: 32). They reveal information on plants' carbon allocation and soil foraging patterns as well as their adaptability to local climates and environments. Root system architecture, represented in part by the last two traits, is concerned with the spatial distribution and structure of individual root system components. It determines mechanical properties of the root system, interactions between root and soil, and within-root transport processes (Freschet, Pagès *et al.* 2021: 1013, 1021).

Root diameters are typically given as means of diameters, either of the whole root system (Ianucci & Amato 2021: 3208; Luo *et al.* 2020: 4) or for specified root orders (Xing *et al.* 2024: 3; Ye *et al.* 2024: 3). They have traditionally been used for root classification, typically differentiating between coarse roots (> 2 mm diameter) and fine roots (≤ 2 mm diameter), with the latter category being assumed to primarily serve nutrient uptake functions. However, this classification fails to account for structural and functional differences within the group of roots ≤ 2 mm, and it neglects the chance for fourth- or higher-order roots, which are ill suited for substance uptake, to occur in this group (Freschet, Pagès *et al.* 2021: 985; McCormack *et al.* 2015: 505; B. Wang *et al.* 2023: 2873). Smaller diameter cutoffs, for example ≤ 0.5 mm in *Pinus sylvestris*, have been shown to be more useful for functional categorisations of fine roots (Mucha *et al.* 2020: 512). However, due to the ontogenetic, intraspecific, and very large interspecific variability of root diameters, order-based diameter values are the most favourable choice to ensure data comparability across studies (Freschet, Pagès *et al.* 2021: 1022; McCormack *et al.* 2015: 507; McCormack *et al.* 2017: 30). Greater diameters, especially of first-order roots, are associated with efficient soil penetration but slower resource uptake and use. Large diameters of order 1, 2 and 3 roots are indicative of plants' reliance on mycorrhizal symbiosis for substance uptake, while thinner absorptive roots are connected to acquisitive strategies independent of mycorrhiza (Addo-Danso *et al.* 2020: 7; Bergmann *et al.* 2020: 3; Fort & Freschet 2020: 1573; Freschet, Roumet *et al.* 2021: 1129).

Specific root length (SRL) and *specific root area* (SRA) describe the length and surface area of a root per unit of dry mass. They measure roots' capacities for soil exchanges against their carbon costs and are thus considered key characteristics for root economics and resource acquisition strategies (Bergmann *et al.* 2020: 1; Freschet, Pagès *et al.* 2021:

1025). Particularly SRA is currently considered the most direct gauge of how much root-soil interactive surface is available to a plant relative to the biomass invested in root formation (Langguth *et al.* 2024: 1427). While both SRL and SRA can only function as approximations for the amount of soil influenced by absorptive roots (Freschet, Roumet *et al.* 2021: 1134), high values in these traits indicate high resource acquisition capacities, particularly if they occur together with low diameters of first- to third-order roots (S. Wang *et al.* 2021: 82). Resource conservation strategies, in contrast, are expected to result in reduced root expansion (Torres *et al.* 2022: 168). SRL and SRA are assumed to be consistent across time within an individual plant but may show some variation within a species across different environments (McCormack *et al.* 2017: 30). Both are frequently used traits for morphological root analyses (Alsajri *et al.* 2020: 250; Xing *et al.* 2024: 3; Ye *et al.* 2024: 3).

Root tissue density (RTD) is calculated by dividing the root dry mass by the volume of fresh roots collected. It enables an estimation of carbon investment required for root construction (Freschet, Pagès *et al.* 2021: 1027), with higher densities indicating higher carbon costs, but also an increased lifespan and mechanical durability of the roots (Freschet, Roumet *et al.* 2021: 1129). RTD is thought to be stable within a species across different environments, root ages, and vegetation periods (McCormack *et al.* 2017: 30).

Root dry matter content (RDMC) is determined via dividing root dry mass by root fresh mass. Like RTD, RDMC depends on root anatomy (e.g., cell wall thickness and lignification, or the presence of heavy storage tissues) (Vescio *et al.* 2020: 9) and provides insight into the carbon costs required to build a unit of root, irrespective of its water or air content. Both measures complement each other in distinguishing conservative from acquisitive root formation strategies. High RTD and RDMC are considered markers of resource conservation, low nutrient uptake, or plants' adaptation to stress, while low RTD and RDMC indicate fast expansion and high resource uptake capabilities (Freschet, Pagès *et al.* 2021: 1027; Freschet, Roumet *et al.* 2021: 1142), especially in unit with high SRL and SRA. Some measure of root mass, be it RTD or RDMC, is assessed in virtually all investigations of root morphology (Alsajri *et al.* 2020: 250; López *et al.* 2021: 1708; Vescio *et al.* 2020: 3; Xing *et al.* 2024: 3; Ye *et al.* 2024: 3).

Root branching density (RBD), the number of lateral roots per unit of parent root length, is part of the root system architecture trait complex. It relates to a plant's overall number of roots, determines the frequency of branching occurrences across

the sample, and is highly variable both within and between species (McCormack *et al.* 2017: 30). Intraspecific variability in RBD may stem from plants' local adaptations to resource availability and acquisition, as RBD varies with soil resource patchiness (Freschet, Roumet *et al.* 2021: 1125). High RBD signifies intensive utilisation of high, localised nutrient concentrations, typically coinciding with the production of thin roots. Low RBD, in contrast, relates to lower nutrient availability and roots' consequential tendency to explore larger soil areas (Freschet, Pagès *et al.* 2021: 1017; Li *et al.* 2017: 1140). Additionally, high RBD stabilises soil aggregates and, especially if aligned with high SRL, improves plant anchoring in the soil by increasing the physical force required to uproot a plant (Freschet, Roumet *et al.* 2021: 1132, 1136).

The *number of root tips per dry weight* (NRT/DW) is somewhat rarely investigated (e.g., in Ficken & Wright 2019: 67), likely due to the invasive nature of many sampling techniques often resulting in damage to root tips. However, the tips rank among the most physiologically active parts of the root and feature the highest nitrogen concentrations of all root tissues (Freschet, Pagès *et al.* 2021: 1038, 1080; Iversen *et al.* 2017: 22). Their examination thus promises insights into root nitrogen uptake and metabolic activity. Where they are considered, usually the absolute number of root tips occurring in a sample is given (Alsajri *et al.* 2020: 250; Ianucci & Amato 2022: 3208; Jiao *et al.* 2022: 3; Luo *et al.* 2020: 4). NRT/DW is related to RBD as another measure describing the branching patterns of a root, providing additional information on root system architecture.

1.2 Linking roots with aboveground dynamics

While the morphology and architecture of roots are relatively well-described, less is known about their connections beyond the soil surface. Root development is likely determined by exogenous factors regulating root growth both directly (e.g., soil temperature, moisture, and nutrient contents) and indirectly (e.g., air temperature), as well as endogenous factors directly influencing root development from within the plant (e.g., photosynthetic activity, carbon and nutrient storage, plant growth regulators) (Radville *et al.* 2016: 3623). Roots, in turn, are known to influence the growth and development of aboveground plant organs (Weemstra, Kuyper *et al.* 2023: 8). However, knowledge specifically on how root traits are linked to weather, for example, or to the phenological development of the shoot, is limited to partial and somewhat inconclusive explorations of these associations.

1.2.1 Roots and weather

Among all weather variables, the link between root traits and soil or air temperature has likely been the most thoroughly researched, especially in crop plants. In both lab and field settings, close correlations have been found between temperature and some morphological and architectural traits, while other traits seem to be independent of temperature (J. Wang *et al.* 2021: 1859). In *Mimosa sepiaria*, for example, root length and diameter increased with temperature, and *M. sepiaria* and *Corchorus capsularis* showed a strong increase in RBD following a rise in temperature (Luo *et al.* 2020: 9). In soybeans, as well, the number of root tips, along with root mass, length, volume, and surface area, was found to strongly correlate with temperature (Alsajri *et al.* 2020: 252). Contrastingly, the origination of lateral roots, a trait related to root branching density, has been observed to be independent of temperature in soy (Torrion *et al*. 2012: 1708). In maize, heat stress had opposing effects on RTD and RBD of primary and seminal roots (Vescio *et al.* 2020: 3-4). In other plants and settings, temperature explained only a small part of variations in root form or could not be shown to influence them at all (Abramoff & Finzi 2015: 1058; Radville *et al.* 2016: 3622). Overall, root growth can be expected to be promoted by temperatures within a plant's optimum range, whereas too high or low temperatures will reduce root carbon allocation and development (Alsajri *et al*. 2020: 252; Calleja-Cabrera *et al.* 2020: 6; Karlova *et al.* 2021: 1062; Luo *et al.* 2020: 5, 9). A lack of correlations occurring in individual studies may be due to a selection of temperature ranges that are too narrow, failing to extend beyond the optimal range. Environmental factors also interact in shaping root traits, potentially leading to variations in root formation strategies (Freschet, Roumet *et al.* 2021: 1134) that are challenging to detect.

The effects of precipitation on root morphology and architecture are less thoroughly studied than the impact of temperature. Previous findings reveal a similarly unpredictable pattern of correlations between altered precipitation regimes and root traits as is the case for temperature. Herbaceous and woody plants likely differ in their adaptability to reduced precipitation, with herbs being more capable than trees of efficiently allocating biomass to the roots when faced with drought stress (Xing *et al.* 2024: 7). However, not all woody species show the same patterns, either. For example, for *Fagus sylvatica* and *Picea abies*, contrasting strategies in fine-root adaptations have been observed during severe droughts (Nikolova *et al.* 2020: 9).

As a reaction to reduced precipitation, a decrease in root production and, in some cases, absolute root biomass has been observed in herbaceous plants (Slette *et al.* 2023: 5; P. Wang *et al.* 2020: 1496; Xing *et al.* 2024: 8). At the same time, biomass gains of roots relative to shoots, which occur in various plant growth forms during droughts, indicate enhanced efforts to invest in roots under low precipitation conditions (Larson & Funk 2016: 833; Sun *et al.* 2022: 9; Xing *et al.* 2024: 7). Such reactive shifts of biomass allocation towards the roots are larger where the climate is particularly arid, suggesting plants' increased investment in root growth in dry areas. Wherever this is not possible for herbaceous plants, growth of thinner roots with a larger soil-root interface has been hypothesised (P. Wang *et al.* 2020: 1496-1497; J. Wang *et al.* 2021: 1857, 1863) to maximise water uptake capacities relative to the root biomass plants can form. Correspondingly, a negative correlation with precipitation has been identified for first-order root length, particularly of species with comparatively thin roots (Li *et al.* 2017: 1144). This suggests that lower precipitation is associated with longer first-order roots, resulting in larger root surface areas. However, a contrasting effect of increasing precipitation correlating with high SRL and low diameters has been observed by Larson and Funk (2016: 830). Yet another outcome has been described by Slette *et al.* (2023: 5-6), who have found no significant changes whatsoever in diameters, SRL, or RTD under drought conditions in grassland roots. This is also reflected in their finding of two subsequent droughts affecting root biomass production more negatively than just one drought, indicating a lack of adaptations to low precipitation during the first drought event. These varied findings illustrate how herbaceous plants' root form adjustments to changes in precipitation show no clear or consistent pattern. There may be several different, possibly species-specific pathways of how plants react to drought (Larson & Funk 2016: 830; Slette *et al.* 2023: 7).

Atmospheric humidity appears to have secondary effects on roots, modulating the severity of plants' reactions to changes in precipitation. For herbaceous plants, for example, stronger effects of precipitation on root growth have been observed in humid than in dry areas (Xing *et al.* 2024: 7). Additionally, high humidity has been found to co-occur with high RTD, likely reflecting plants' reactions to the vapor pressure deficit changing with fluctuating humidity (Fort & Freschet 2020: 1571, 1573). Vapor pressure influences stomatal transpiration, metabolism in the leaf, and water and nutrient uptake from the soil (Koehler *et al.* 2023: 4790) and is likely to consequentially affect root form as well. A high vapor pressure deficit, for instance, has been shown to correlate positively with root volume, surface area, and average diameter in a tomato

cultivar (Du *et al.* 2020: 6). In contrast to this, high vapor pressure deficit was associated with lower root length, volume, surface area, and root tip numbers in a different setting using tomatoes (Jiao *et al.* 2022: 6). Root dry mass has been observed to be independent of vapor pressure deficit (López *et al.* 2021: 1711).

Overall, several factors associated with temperature and precipitation influence root growth in various ways. What remains unclear are governing principles and patterns of impact, which are essential for reliable predictions of specific root traits' development in relation to weather conditions. The inconsistencies detailed above likely originate from a multitude of differences between prior studies, including their settings chosen, species and growth forms examined, levels of organisation scrutinised, and root entities observed. While such desirable variations are conducive to expanding scientific knowledge on the topic, previous investigations unfortunately also differ in their sampling times and methods chosen, and in their trait calculations employed. Particularly these last factors underline the importance of standardisation in trait definition and data collection, as recommended by Freschet, Pagès *et al.* (2021: 975-976), to achieve comparable results.

1.2.2 Roots and shoots

Root growth constitutes a carbon investment for plants, both for their construction as well as for ongoing respiratory processes in the standing root biomass. Hence, root development depends on carbon that has been photosynthetically assimilated in the shoots (Abramoff & Finzi 2015: 1058). The resulting trade-off in above- and belowground carbon allocation is commonly represented as some ratio of root to shoot or to whole-plant biomass, a frequently used parameter (Alsajri *et al.* 2020: 254; Iannucci & Amato 2021: 3211; Iversen *et al.* 2017: 19; Luo *et al.* 2021: 4; Torres *et al.* 2022: 174) whose behaviour with changing plant size or environmental conditions is relatively well-understood (McCormack *et al.* 2017: 29). This ratio has previously been considered a reasonable approximation of plants' investment in aerial versus subterranean organs. It represents a holistic view on assimilate distribution across all belowground plant parts as opposed to the aboveground organs (Klimešová *et al.* 2018: 2119). However, it cannot represent small-scale variations in root morphology and metabolic activity, or capture targeted allocations of carbon to specific root functions (McCormack 2017: 30). It also fails to account for root respiration rates, which are notoriously difficult to measure but crucial for accurately estimating investment distribution (Freschet, Roumet *et al.* 2021: 1144; Klimešová *et al.* 2018: 2121). Therefore, ratios of root dry matter to shoot or whole-plant dry matter are a poor proxy for

the actual distribution of carbon to individual plant organs, often leading to an underestimation of the energy allocated to the roots (Kong & Fridley 2019: 1632) and necessitating alternative descriptions of root-shoot balancing.

The trade-off between above- and belowground growth has been linked to a delay in root development relative to aboveground phenology. In trees, shrubs, and herbaceous plants of subtropical to boreal biomes, peak root growth has been found to lag behind shoot growth by 25 ± 8 days (Abramoff & Finzi 2015: 1056-1058). Another analysis, which considered forest and grassland vegetation separately, showed peak root production to be delayed compared to leaf-out times by 45 days for forests and 15 days for grasslands (Steinaker & Wilson 2008: 1222). Though asynchronous, root and shoot growth seem proportional to each other, with reductions in root biomass accumulation correlating with plant senescence in late summer and autumn (Abramoff & Finzi 2015: 1056-1058; Alsajri *et al.* 2020: 254). Although temporal relationships of root and shoot phenology are thus not entirely uncharted, still more information is required on the links between above- and belowground phenology (Piao *et al.* 2019: 1934-1935).

Additionally, there is virtually no research exploring possible links of shoot phenology to root form. In the only such study conducted so far, which scrutinises links of morphological and architectural root traits with the phenological stage of senescence, end-of-season above- and belowground phenology were found to correlate with each other as well as with the dry matter contents of leaves and roots. The results indicated a high RDMC to be the best predictor of plant survival during winter. At the same time, root and leaf tissue densities likely coordinated above- and belowground phenology, while aboveground plant height possibly influenced architectural root traits (Ye *et al.* 2023: 9-11). A thorough search of relevant literature has revealed no further information on the topic.

Overall, root development is highly species-specific (as illustrated, e.g., in Luo *et al.* 2020: 6; Palacio & Montserrat-Martí 2007: 528-529; Torres *et al.* 2022: 173) and depends heavily on the prevailing environmental conditions (Radville *et al.* 2016: 3622). Much of the aboveground initiators, drivers, and limitations of roots' morphological and architectural development are still subject to speculation. The high variability of root form within and between species is possibly the most consistent result across all studies exploring such relationships. General deductions on the interrelation of root and shoot development across species are, if at all possible, a distant prospect.

1.3 Herbaceous phenology and PhenObs

Interest in aboveground phenological development has increased in recent years following renewed scientific attention to phenology as a bio-indicator for climate change (Intergovernmental Panel on Climate Change 2023: 49; Menzel *et al.* 2020: 2600). Yet, the scope of phenological investigations remains somewhat narrow, with most studies focusing on woody plants or crops, while data on herbaceous plants remains limited (Nordt *et al.* 2021: 822). To alleviate this deficit, in 2017, the German Centre for Integrative Biodiversity Research (iDiv 2024) in Jena launched PhenObs, an international network of botanical gardens monitoring herbaceous plants' phenology. This network, in which the University of Vienna (2024) participates, documents variations in plant phenology to be able to understand and predict effects of biotic and abiotic factors on plant development, and to derive conclusions on species' adaptability to changing environments. This is achieved by measuring phenophases using a standardised protocol capturing the most significant developments in plants' life cycles (Nordt *et al.* 2021: 824, 829-30).

In a subproject of PhenObs carried out from 2019 to 2023, plant mix beds were set up in the botanical gardens of Berlin, Halle, Leipzig, Jena, Vienna, and Chicago. These beds featured a standardised soil mixture, were exposed to similar light conditions and treatments, and harboured multiple individuals of ten different herbaceous species to be monitored over the course of several years. The species pool included *Anthericum ramosum, Astragalus onybrichis, Campanula glomerata, Dianthus gratianopolitanus, Galium glaucum, Linaria genistifolia, Linum austriacum, Salvia nemorosa, Sanguisorba minor,* and *Scabiosa canescens.* To ensure uniformity across the locations, all individual plants were propagated from a single original population per species and distributed to the botanical gardens. Thusly equipped, the mix beds were intended as a benchmark for the comparability of data collected in the various botanical gardens, including measurements within the larger framework project. Of the plants observed in Vienna, *Salvia nemorosa* and *Sanguisorba minor* showed the most consistent phenological development across all individuals of the species (Narazi-Montazer 2023: 10-14; Weiwar 2023: 22, 38), indicating low intraspecific variability. Variations in their phenology are therefore likely driven primarily by external factors, rendering these species particularly suitable for a comparison across Berlin, Halle, Jena, and Vienna, where they were grown and monitored. The mix beds were discontinued in December 2023, which afforded an opportunity to collect data on the phenologically observed plants' root systems.

1.4 Known root characteristics

As temperate, perennial hemicryptophytes (Kutschera *et al.* 1992: 279, 625), *Salvia nemorosa* and *Sanguisorba minor* survive adverse conditions outside of the growing season by discarding most of their aboveground growth and remobilising carbon into their roots, which are embedded in a protective layer of soil. They reemerge in spring from basal parts of the previous year's shoots or from belowground buds buried in shallow soil (Fischer *et al.* 2008: 109). Root growth initiation and aboveground budding therefore likely depend on soil temperatures surrounding the coarse roots, as well as their amount of carbon stored (Steinaker & Wilson 2008: 1226-1227). Large carbon reserves may enable plants to invest in root growth prior to shoot growth in spring, increasing their competitive abilities in the exploitation of water or scarce resources while leaf growth is still inhibited by low temperatures (Radville *et al.* 2016: 3623). Further growth above and below the ground during the progressing vegetation period depends on photosynthate availability, with root growth likely being modulated by shoot growth (Abramoff & Finzi 2015: 1058). Due to low temperature and carbon availability, root growth may slow down in autumn and winter but is unlikely to cease completely (Radville *et al.* 2016: 3624; Steinaker & Wilson 2008: 1223). Specific characteristics of the root morphology, system architecture, and development of *Salvia nemorosa* and *Sanguisorba minor* are discussed in detail below.

1.4.1 Salvia nemorosa

The root system of adult *Salvia nemorosa* individuals has been described as typically consisting of a winding, woody tap root that can reach up to 15 mm in diameter and 230 cm in length, with up to ten lateral roots branching off within 5 cm segments. Adventitious or shoot-borne roots can occur in older individuals but remain subordinate to the tap root. Overall, the widely ramified root system possesses a cylindrical shape (Kutschera *et al.* 1992: 624). Closer descriptions of root morphological and architectural traits are unavailable for *S. nemorosa,* but other species of the genus have been scrutinised in more detail. For *S. hispanica,* average diameters of the whole root system were found to range between 0.25 and 0.37 mm in juvenile plants. SRL ranged from 213.8 to 337.6 mm mg^{-1} and showed strong negative correlations with shoot traits (Ianucci & Amato 2022: 3209-3212). Interpreted with caution, these values provide a coarse reference for root traits of the genus. Unfortunately, most other studies that include form-related root traits of *Salvia* do so qualitatively or without specifying the orders and amount of root material for which the traits were measured (e.g., Burnett *et al.* 2005: 776; Khoshsokhan *et al.* 2022: 2), thereby severely limiting their usefulness for comparisons.

Fine root phenology has been investigated in *S. lavandulifolia,* for which asynchronous root and shoot growth have been recorded. Fine root growth shows a moderate peak in spring following shoot growth, is then reduced during summer, and exhibits a maximum in autumn. Continued development of fine roots during the winter months (Palacio & Montserrat-Martí 2007: 528-529) supports the assumption that the growth of deep-reaching roots, such as those of various *Salvia* species, may be relatively independent of air temperature (Radville *et al.* 2016: 3625). Continuous root growth is likely also aided by the ability of *S. nemorosa* to store large amounts of assimilated carbon in the tap root and its most basal laterals (Kutschera *et al.* 1992: 625).

1.4.2 Sanguisorba minor

Sanguisorba minor possesses a cylindrical or frustoconical root system, with a tap root up to 160 cm in length and 2-8 mm in diameter. Within 2 cm sections of the tap root, up to eight lateral roots can branch off and ramify further. Often, three to six of the uppermost lateral roots grow particularly thick horizontally before bending and extending downward (Kutschera *et al*. 1992: 279). While *S. minor* possesses deep-reaching primary roots whose carbon allocation increases with plant age (Torres *et al.* 2022: 167), larger parts of the root system are closer to the surface than is the case for *Salvia nemorosa*. They might thus be more directly affected by air temperature (Radville *et al.* 2016: 3625).

Average SRL of *S. minor* has been observed to range around 262 to 319 mm mg^{-1} in watered and unwatered plants, respectively (De Lillis *et al.* 2005: 217). The lower SRL in watered relative to unwatered plants is likely due to higher diameters and tissue densities occurring in the roots of watered individuals. Lower RTD values, in contrast, may be caused by aerial canals identified in the place of collapsed phloem tissue in coarse roots of *S. minor* (Tocai Moţoc *et al.* 2023: 4-5). Such air-filled canals can reduce the root mass without affecting diameters, thus impacting density. RDMC for juvenile *S. minor* has been observed to be around 0.11 mg mg^{-1} in lateral to 0.28 mg mg^{-1} in primary roots, which is an increase from seedling RDMC at 0.08 to 0.21 mg mg^{-1}, respectively. Similarly, there is an increase in root branching orders from two in seedlings to five in juvenile plants (Torres *et al.* 2022: 172-175). This prompts the assumption of a possibly even higher RDMC as well as a further increase in root orders for adult plants, but must be considered with caution, since trait plasticity is high in *S. minor* (De Lillis *et al.* 2005: 218).

Some correlations between above- and belowground traits in *S. minor* have been reported. In Torres *et al.* (2022: 174-178), SRL of the tap root was negatively correlated to total leaf area and total shoot biomass, accounting for a significant portion of these

traits' observed variation. This possibly indicates competition in carbon allocation between roots and shoots. At the same time, a positive relation of specific leaf area with tap root SRL suggests some coordination of above- and belowground development.

1.5 Research questions

Insights into the form of absorptive roots of *Salvia nemorosa* and *Sanguisorba minor* beyond those outlined above are presently unavailable. To add to the pool of knowledge on these species' fine root morphology and architecture, as well as their link to aboveground phenology and weather influences, this thesis therefore investigates their roots based on the following questions:

1. What are defining morphological and architectural characteristics of the absorptive fine roots of *Salvia nemorosa* and *Sanguisorba minor* growing in the botanical gardens in Berlin, Halle, Jena, and Vienna?
2. How do these plants' root traits relate to the weather conditions present across the different sites and years?
3. How do the root traits relate to the aboveground phenological development of *Salvia nemorosa* and *Sanguisorba minor*?

2 Methods

2.1 Project context

The data for this thesis was gathered within the mix bed subproject of PhenObs outlined in Section 1.3, and includes measurements collected for *Salvia nemorosa* and *Sanguisorba minor* in the botanical gardens of Berlin, Halle, Jena, and Vienna. The mix beds were positioned in shadeless areas and filled with a soil mixture containing lime, sand, and marl that is characteristic for the Pannonian Basin, for which around half of the species observed in the project are typical. In each location, the beds were divided into five identical plots which served as simultaneous repetitions to increase the sample size. The square plots, measuring 2.56 m² each, were then planted with 1-2 individuals of each of the various species to be observed. While species composition varied slightly among the botanical gardens, within one location, each plot contained the same number of species and individuals, which were distributed randomly to achieve different spatial compositions of species across the plots. The configuration of the Viennese mix bed during summer 2023 is illustrated in Figure 2.

To ensure comparability of the phenological observations, phylogenetic variation between the individual plants of a species growing in the different locations was minimised by propagating them from a single original population and shipping them to the participating gardens. Common monitoring protocols were used across all sites, with the observation focusing on the individuals planted in the beds at the start of the project. In some instances, however, these plants died, requiring an adaptation of the monitoring. In cases where two individuals had been planted or the dead individual had already reproduced,

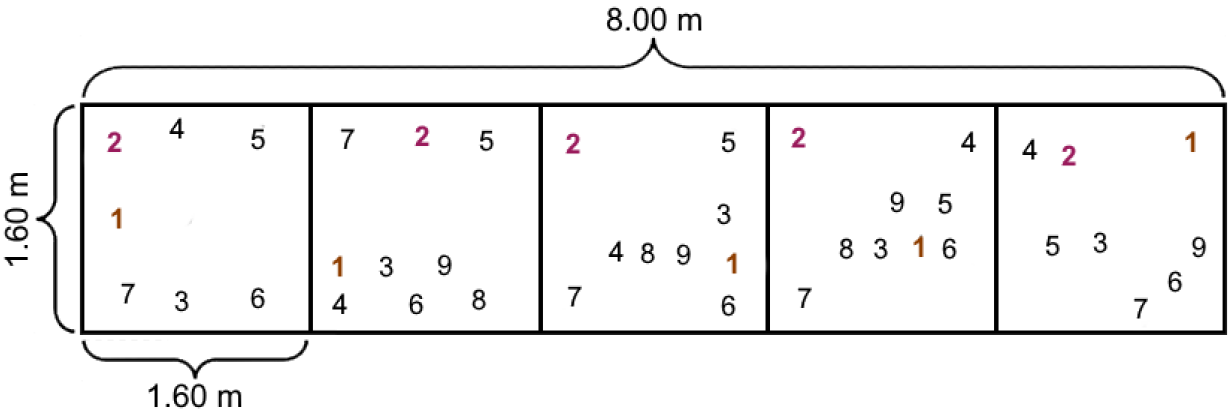

Figure 2 Schematic illustration of the Viennese mix bed. Depicted is the distribution of plants during spring and summer 2023 (data from Weiwar 2023: 11). Numbers denote species as follows: 1 *Salvia nemorosa* (orange), 2 *Sanguisorba minor* (pink), 3 *Linum austriacum*, 4 *Galium glaucum*, 5 *Dianthus gratianopolitanus*, 6 *Anthericum ramosum*, 7 *Linaria genistifolia*, 8 *Scabiosa canescens*, 9 *Campanula glomerata.* Missing numbers in a plot indicate plants having died without being replaced.

another individual of the species within the same plot was chosen as the new focus for the phenological observation. The points in time of such changes in monitored individuals were not recorded. Interventions in the plots were kept to a minimum, with watering limited to what was absolutely necessary for plant survival during drought and heat. Pruning was done solely to prevent individuals from overgrowing the plot or other monitored plants. The occasions and frequency of such disturbances were not recorded. The plants were allowed to grow in the plots from spring 2019 to the end of 2023, when the mix beds were discontinued.

2.2 Data collection

2.2.1 Phenological observations

The phenological development of *Salvia nemorosa* (Figure 3A, C) and *Sanguisorba minor* (Figure 3B, D) was continuously measured over the project duration. The common monitoring protocol included recording the days of year in which initial growth (IG), first leaf (FL), first flower (FF), peak flowering (PF), end of peak flowering (EPF), end of flowering (EF), first fruit (FFr), start of senescence (SS), and 50% senescence (S50) occurred. The collected data are available on request from the central coordination of the project in Jena (iDiv 2024) and were provided for this thesis for the years 2020 to 2022. From these observations, flowering duration (FD) and vegetation period (VP) were calculated and included in the analysis. FD was defined as the number of days from first flower to the end of flowering, and VP was calculated as the number of days between first leaf and the start of senescence, as these represent the earliest and the latest consistently observed phenophases. An overview of the phenological variables used is listed in Table 1.

2.2.2 Root measurements

As part of the discontinuation of the mix beds at the end of 2023, the root systems of the plants monitored throughout the previous years were unearthed, washed, and analysed following a standardised procedure across all participating gardens. Harvesting took place from mid November to mid December and was carried out plot by plot using common spades and digging forks. In the Viennese mix bed, smaller individuals with superficial roots were harvested first, while large specimens and species with deep-reaching tap roots were dug out last to avoid damaging the smaller plants' roots. Though the aim was to excavate the individuals' root systems as entirely as possible, for some plants, especially *Salvia nemorosa*, the more deep-seated root sections could not be recovered. In early December, a forecast of heavy snowfall during the ongoing harvest

necessitated the pre-emptive transfer of those plants still remaining in the bed to a frost-free area to enable the processing of the roots at a later point in time. The roots were dug up along with the adhering soil to prevent desiccation, transferred into plastic bags, and stored in an unheated greenhouse on the premises of the botanical garden.

For processing, the roots were soaked in cold water for 15-20 minutes before being rinsed in a sieve until no more soil adhered to the fine roots and tips. Thusly prepared, smaller sections were cut from the roots which then, using a stereo microscope, were searched for intact segments consisting of first, second, and third order roots. Those were separated from the higher order roots and collected in a water-filled petri dish. Once enough material had been obtained from an individual, the root cuttings were dried on filter paper for 15 minutes before being weighed, packed in tubes, and frozen for interim storage.

The frozen samples of the participating gardens were shipped to Jena, where they were thawed, distributed evenly across a transparent container (York 2020) within a thin layer of water, and scanned using an adapted Epson Expression 12000XL flatbed scanner with transparency unit. Instructions for reproducing the device adaptations are available from York (2023), and example sections of root scans can be found in Figure 3C and D for both species. The scans were analysed with RhizoVision Explorer (version 2.0.3, Seethepalli & York 2021). In accordance with suggestions in the user manual (Seethepalli *et al.* 2021: 5-7), for both species, analysis mode was set to broken roots, conversion of pixels to physical units was activated with a resolution of 1200 DPI, image thresholding level was set to 210, and non-root objects smaller than 1 mm² were filtered. Root pruning was enabled, but due to the different structures of the roots of *Salvia nemorosa* and *Sanguisorba minor,* individual settings for the species were necessary. For the thicker roots of *Salvia nemorosa,* the pruning threshold was set to 8 pixels, and edge smoothing was enabled at level 4 to reduce the software's overestimation of branch points. For *Sanguisorba minor,* the root pruning threshold was set to 4 pixels, and edge smoothing was disabled. Though one of the strengths of RhizoVision Explorer is its ability to calculate data separately for individual sections of scanned roots according to their diameters, this function could unfortunately not be used to gain insights into specific root orders. In both species, the diameters of order 1, 2, and 3 roots did not differ enough to be used for a reliable distinction between the root orders.

The roots' fresh weight was ascertained during sampling. Root properties measured from the scans by RhizoVision Explorer included total root length, total root volume, root surface area, number of root tips, root branching density (RBD), as well as average (D_{avg}), median (D_{med}), and maximum (D_{max}) diameter. After scanning, the roots were dried for 72 hours at 70°C and allowed to cool down in an exsiccator before their dry weight was measured. The amount of root matter collected per plant differed due to their variations in size. Between 52 and 697.1 mg of fresh root material were harvested for individuals of *Salvia nemorosa* in the various botanical gardens, which, after drying, amounted to between 15.5 and 184.5 mg dry weight. For *Sanguisorba minor*, the material collected ranges from 24 to 175 mg fresh weight, and 4.7 to 38.1 mg dry weight, respectively. To counteract distortions due to these different root material amounts, root dry matter content (RDMC), root tissue density (RTD), specific root area (SRA), specific root length (SRL), and the number of root tips per dry weight (NRT/DW) were calculated from the measurements above. For the analysis, only these parameters independent of the amount of sampled material were used, whereas the total measurements were disregarded. Table 1 in Section 2.3 summarises the root parameters used in the analysis.

2.2.3 Weather data

Meteorological measurements were obtained from weather stations as close as possible to the respective botanical gardens. For the German cities, data are publicly available from the DWD Climate Data Center (2024), while for Vienna, they are provided by the GeoSphere Austria data hub (2024). From both services, daily data were retrieved on average temperature, maximum temperature, amount of precipitation, relative humidity, and vapor pressure, with most of these measurements having been collected at a height of 2 metres above ground level. In Berlin, the meteorological station closest to the botanical garden is Berlin Dahlem, with a linear distance of around 500 metres. For Halle, the closest meteorological station providing a complete set of measurements for the parameters listed above is Bad Lauchstädt, with a linear distance of around 12,400 metres. For Jena it is Jena Sternwarte, with a linear distance of around 200 metres, and in Vienna the closest weather station to the botanical garden is Wien Innere Stadt, with a linear distance of around 1,900 metres. The weather data was acquired for 2020 to 2023, to match the three years of phenological observations and to include the year of the root data collection.

To match the finely scaled daily weather data to the root measurements available for only a single point in time, annual precipitation sums (PRCP) and yearly averages for average temperature (T_{avg}), maximum temperature (T_{max}), relative humidity (RH), and vapor pressure (VP) were calculated. To compensate for this coarse resolution of measurements, three additional variables capturing differences between the locations on a finer scale were devised. Firstly, temperature intervals with a range of 5°C were established, and for each interval, the annual number of days on which the maximum temperature fell within its range was ascertained. Because root growth stops below a certain temperature threshold (Alvarez-Uria & Körner 2007: 211), the days with maximum air temperature below 0°C were summarised into one interval. Second, the number of days per year where no precipitation occurred was counted. Finally, for each year and location, the duration of the longest streak of days without any precipitation was determined. The first of these additional variables detects differences in temperature distributions between the years and locations, while the latter two capture drought periods. Along with the abovementioned weather variables, these numbers of days with maximum daily temperature within a 5°C interval (e.g., Days 0-4.9°C), numbers of days without precipitation (DWP total/year), and longest streaks of days without precipitation (DWP) are outlined in Table 1.

2.3 Summary of sample and procedures

Across all locations, the roots from 20 individuals of *Salvia nemorosa* and 19 individuals of *Sanguisorba minor* were harvested. This included five individuals from each of the four locations of Berlin, Halle, Jena, and Vienna. One *S. minor* growing in Halle had died prior to the harvest. This produces a set of 39 plants for which the relationship of root form with phenology and weather can be assessed. These plants' phenology was monitored for three consecutive years from 2020 to 2022, resulting in an overall sum of 117 phenological observations corresponding to the root measurements that were taken at the end of 2023. Weather data were retrieved for the four years from 2020 to 2023 to encompass the entire period captured by phenological and root measurements. A summary of these variables collected and calculated for the analysis, as well as their abbreviations and units, are listed in Table 1.

In the analysis, the variables of each of the three domains – roots, weather, and phenology – were initially checked for their distribution patterns. For all normally distributed root variables, one-way ANOVA was conducted against both the location and the species as independent factors. For non-normally distributed root traits, a Kruskal-

Wallis test was used instead. This procedure allows for examining how significantly root form differs between or within the sites and species. Analogously, ANOVA or Kruskal-Wallis tests were conducted for weather variables against the location as independent factor, to identify those weather parameters differing most significantly between the gardens. For phenological variables, too, Kruskal-Wallis tests were performed against the location for each species individually. Finally, Spearman's rank was calculated between each root variable and each weather variable, as well as between root traits and phenological observations, to identify patterns of covariation across the three domains. Due to the small sample size of only five individuals per location, these correlations were calculated across the entire sample rather than separately for each garden.

Table 1 Overview of variables used, including path of calculation where applicable.

	Abbr.	Name	Path of calculation	Unit
Roots n = 39	RDMC	root dry matter content	dry weight / fresh weight	mg mg^{-1}
	RTD	root tissue density	dry weight / volume	mg mm^{-3}
	SRL	specific root length	total root length / dry weight	mm mg^{-1}
	RBD	root branching density	number of branch points / total root length	mm^{-1}
	D_{avg}, D_{med}, D_{max}	average, median, and maximum diameter		mm
	SRA	specific root area	total surface area / dry weight	mm^2 mg^{-1}
	NRT/DW	number of root tips per dry weight	number of root tips / dry weight	mg^{-1}
Weather	T_{avg}	averages of mean daily temperatures (°C)	yearly sum of mean daily temperatures / number of days	°C
	T_{max}	averages of maximum daily temperatures (°C)	yearly sum of maximum daily temperatures / number of days	°C
	Days [interval]	numbers of days with maximum daily temperature within a 5°C interval	yearly sum of days < 0°C 0-4.9°C 5-9.9°C 10-14,9°C 15-19.9°C 20-24.9°C 25-29.9°C 30-34.9° ≥ 35°C	days per year
	PRCP	precipitation		mm
	DWP total/year	number of days without precipitation	yearly sum of days with PRCP = 0	days per year
	DWP	longest streak of days without precipitation	highest number of consecutive days with PRCP = 0	
	RH	average relative humidity		%
	VP	average vapor pressure		hPa
Phenology	IG	initial growth		day of year
	FL	first leaf		day of year
	FF	first flower		day of year
	PF	peak flowering		day of year
	EPF	end of peak flowering		day of year
	EF	end of flowering		day of year
	FD	flowering duration	end of flowering − first flower	days
	FFr	first fruit		day of year
	SS	start of senescence		day of year
	S50	50% senescence		day of year
	VP	vegetation period	start of senescence − first leaf	days

3 Results

3.1 Root trait variation across species and sites

Example sections of root scans used to capture the data discussed below are shown in Figure 3. From such images, all root parameters except fresh and dry weight were measured. Table 2 outlines the means, ranges, variability measures, and normality tests for all root traits of *Salvia nemorosa* and *Sanguisorba minor* across all individuals and locations. Among these, RBD is the most stable root parameter. In *Salvia nemorosa,* this trait shows the least dispersion with a CV of 10.9%. All other variables are spread moderately (RDMC, RTD, D_{avg}, D_{med}) to widely (SRL, D_{max}, SRA, NRT/DW) for this species, indicating considerable variation in those traits across the individuals and locations. For *Sanguisorba minor*, similarly, the least dispersed traits include RBD (CV = 12.7%), along with D_{med} (CV = 12.3%) and D_{avg} (CV = 12.9%), which illustrates higher homogeneity in the root diameters than is the case for *S. nemorosa*. All other root traits are highly dispersed in *S. minor.* Shapiro-Wilk tests suggest for a majority of the variables to be normally distributed, though the small sample size might influence this result. Traits apparently deviating from normality are RBD and D_{avg} for *Salvia nemorosa*, and RDMC, RTD, D_{avg}, D_{med}, and D_{max} for *Sanguisorba minor*.

In both species, average and median diameter are strongly correlated ($r = 0.982$, $p < 0.001$). Linear regression, accordingly, suggests most of the variance in one of the variables to be explained by the other ($R^2 = 0.984$, RMSE = 0.011 mm). Given this redundancy, subsequent calculations include D_{avg} only. The maximum diameter is less congruent with the average diameter ($r = 0.746$, $p < 0.001$), with $R^2 = 0.476$ and RMSE = 0.229 mm. Therefore, D_{max} is considered an independent trait and is retained for further analysis.

One-way ANOVA and Kruskal-Wallis tests for root traits of *Salvia nemorosa* against the location reveal significant differences for RDMC ($F = 16.661$, $p < 0.001$, $\eta^2 = 0.758$) and RTD ($F = 15.949$, $p < 0.001$, $\eta^2 = 0.749$). There are no significant differences between the gardens for any other measurements, though the values for SRA reach near-significance ($p = 0.079$). Thus, in *S. nemorosa,* SRL, RBD, D_{avg}, D_{max}, and NRT/DW do not possess any meaningful differences between the locations, as compared to within them. The same process for *Sanguisorba minor* shows significant root trait differences between the gardens only for D_{avg} ($H = 9.651$, $p < 0.022$), suggesting that despite the low dispersion D_{avg} shows in *S. minor,* this trait is subject to location-specific influences. For the other variables, no significant differences between the gardens can be identified. The high

dispersion found in many traits that is outlined above does therefore not coincide with a dependency of these traits on location. The overall values for ANOVA and Kruskal-Wallis

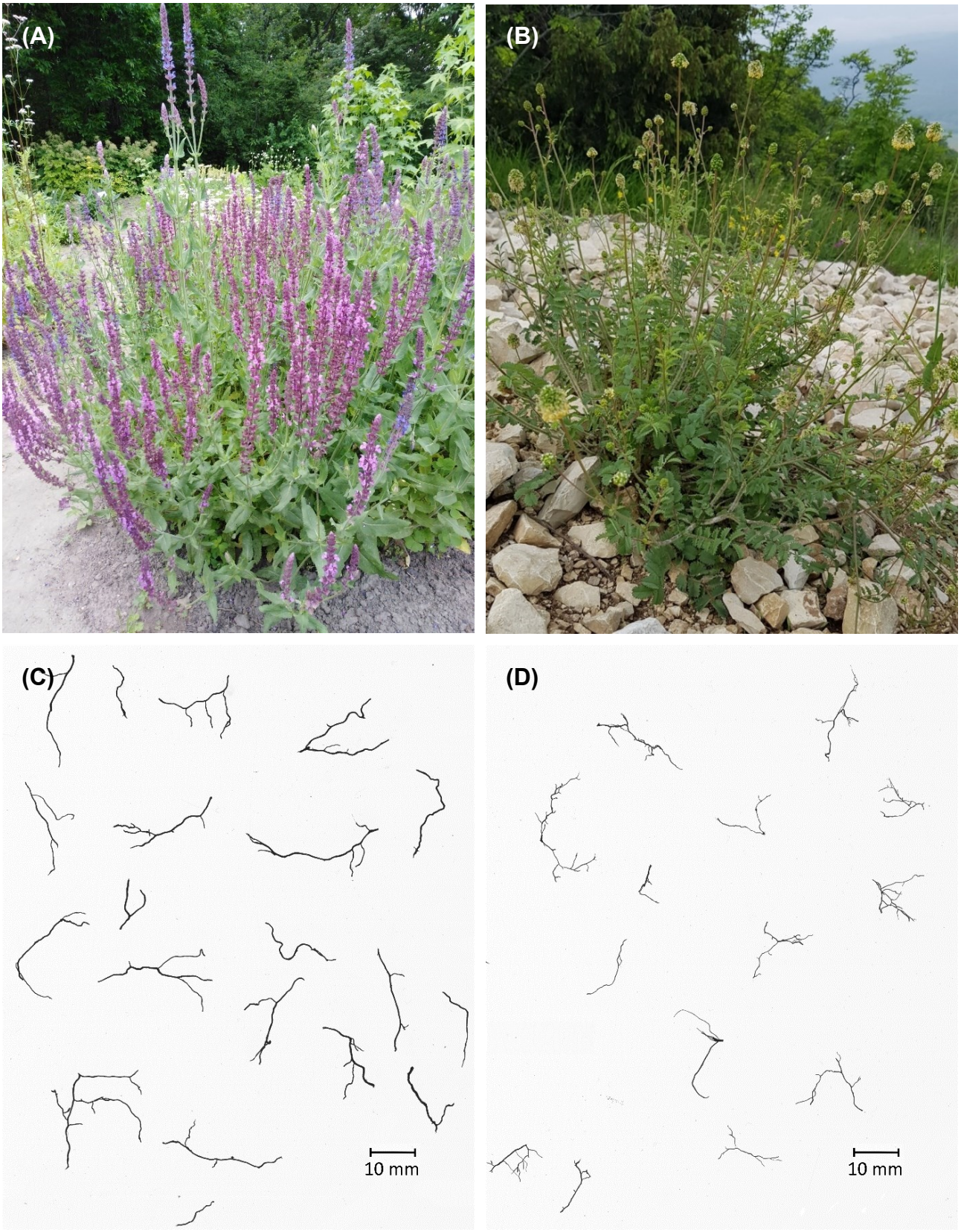

Figure 3 Overview of shoot habitus and root scan sections. Shown are the aboveground appearances of **(A)** *Salvia nemorosa* and **(B)** *Sanguisorba minor* (images in the public domain), along with root scans of Viennese individuals of **(C)** *Salvia nemorosa* and **(D)** *Sanguisorba minor.* The overall size of the scanned area was 210 mm x 297 mm per scan, of which only a section is depicted.

Table 2 Descriptive statistics and normality measures for root traits by species.

Variable	Species	Mean	Min – Max	Std Dev	CV (%)	Shapiro-Wilk	p
RDMC	*S. nemorosa*	0.221	0.163 - 0.298	0.041	18.7	0.943	0.275
	S. minor	0.245	0.126 - 0.507	0.089	36.4	0.837	0.004**
RTD	*S. nemorosa*	0.181	0.130 - 0.247	0.033	18.3	0.959	0.530
	S. minor	0.162	0.096 - 0.310	0.048	29.7	0.868	0.013*
SRL	*S. nemorosa*	41.788	20.079 - 69.535	15.036	36.0	0.954	0.425
	S. minor	121.471	51.892 - 243.238	47.550	39.1	0.942	0.291
RBD	*S. nemorosa*	0.251	0.219 - 0.325	0.027	10.9	0.898	0.038*
	S. minor	0.541	0.404 - 0.680	0.069	12.7	0.949	0.381
D_{avg}	*S. nemorosa*	0.402	0.315 - 0.497	0.067	16.8	0.889	0.026*
	S. minor	0.250	0.214 - 0.320	0.032	12.9	0.890	0.032*
D_{med}	*S. nemorosa*	0.376	0.258 - 0.473	0.070	18.5	0.927	0.134
	S. minor	0.234	0.189 - 0.296	0.029	12.3	0.910	0.073
D_{max}	*S. nemorosa*	1.303	0.898 - 1.779	0.274	21.0	0.955	0.443
	S. minor	0.990	0.648 - 1.803	0.271	27.4	0.872	0.016*
SRA	*S. nemorosa*	50.097	30.003 - 69.954	11.699	23.4	0.968	0.711
	S. minor	92.481	46.277 - 165.742	29.852	32.3	0.965	0.678
NRT/DW	*S. nemorosa*	5.656	1.902 - 12.126	2.565	45.3	0.951	0.382
	S. minor	26.399	13.018 - 49.067	9.367	35.5	0.927	0.151

*p < 0.05, **p < 0.01, ***p < 0.001

Table 3 ANOVA or Kruskal-Wallis results for root traits by location and species.

		ANOVA			Kruskal-Wallis	
Variable	Species	F	p	η²	H	p
RDMC	*S. nemorosa*	16.661	<0.001***	0.758		
	S. minor				3.531	0.317
RTD	*S. nemorosa*	15.949	<0.001***	0.749		
	S. minor				2.785	0.426
SRL	*S. nemorosa*	1.563	0.237	0.227		
	S. minor	1.349	0.296	0.212		
RBD	*S. nemorosa*				4.234	0.237
	S. minor	0.234	0.871	0.045		
D_{avg}	*S. nemorosa*				5.137	0.162
	S. minor				9.651	0.022*
D_{max}	*S. nemorosa*	0.355	0.786	0.062		
	S. minor				0.585	0.900
SRA	*S. nemorosa*	2.725	0.079	0.338		
	S. minor	0.941	0.446	0.158		
NRT/DW	*S. nemorosa*	1.295	0.310	0.195		
	S. minor	1.860	0.180	0.271		

*p < 0.05, **p < 0.01, ***p < 0.001

Table 4 ANOVA or Kruskal-Wallis results for root traits by species.

	ANOVA			Kruskal-Wallis	
Variable	F	p	η²	H	p
RDMC				0.382	0.536
RTD				3.759	0.053
SRL	50.876	<0.001***	0.579		
RBD				28.500	<0.001***
D_{avg}				27.607	<0.001***
D_{max}				10.911	<0.001***
SRA	34.741	<0.001***	0.484		
NRT/DW	91.010	<0.001***	0.711		

*p < 0.05, **p < 0.01, ***p < 0.001

tests against the location can be found in Table 3, and the distribution of those traits that differ significantly between the gardens is illustrated in Figure 4. RDMC and RTD of *S. nemorosa* (Figure 4A, B) are lowest in Berlin, with progressively higher values observed in Halle, Jena, and Vienna. D_{avg} of *S. minor* (Figure 4C), in contrast, primarily varies in how tightly values are clustered within one site, with low dispersion observed in Halle and Vienna and a wider spread in Berlin and Jena.

Similarly, ANOVA and Kruskal-Wallis tests were performed for all root measurements against the species as independent variable, to determine whether root trait variance is larger within or between *Salvia nemorosa* and *Sanguisorba minor*. The resulting values are given in Table 4. RDMC shows no significant difference between the species, and differences in RTD nearly miss significance ($p = 0.053$). All other traits differ considerably and highly significantly ($p < 0.001$) between the two species, pointing towards high interspecific variability in roots. Of those traits that are distinct between the species, lower root diameters in *S. minor* than in *S. nemorosa* suggest the former to possess comparatively thin roots of the first, second, and third order. At the same time, SRL, RBD, SRA, and NRT/DW are considerably higher in *S. minor,* which indicates that this species' thin roots are elongated and highly branched, resulting in a very large surface area relative to root weight compared to *S. nemorosa*.

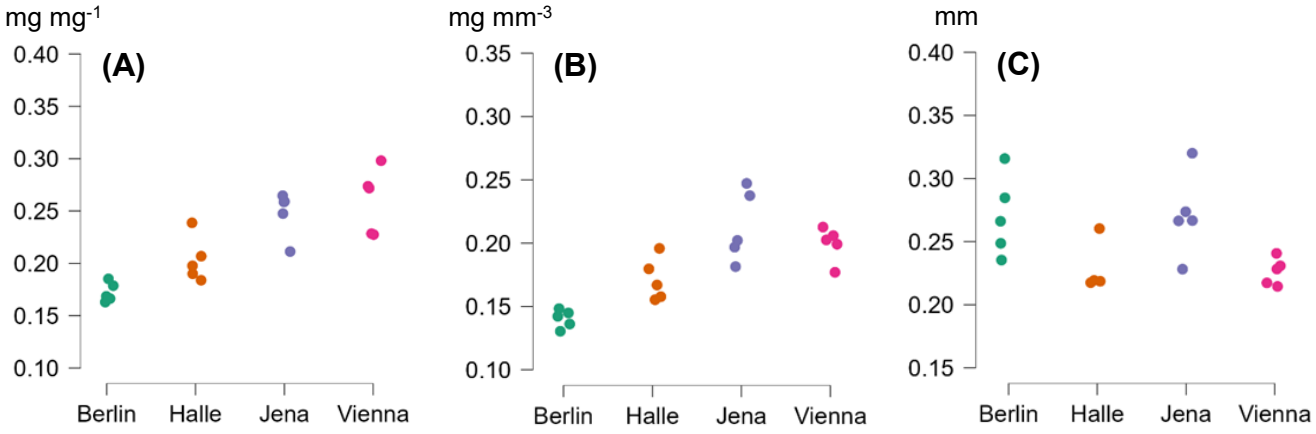

Figure 4 Distributions of the variables that differ significantly between locations. **(A)** RDMC and **(B)** RTD of *Salvia nemorosa*, **(C)** D_{avg} of *Sanguisorba minor*.

3.2 Weather data

3.2.1 Descriptives and variability

As weather is the only location-specific factor influencing plant growth considered in the study design, examining the differences in temperature and moisture parameters across sites is particularly insightful. Generally, Berlin is the coldest location in the sample,

followed in ascending sequence by Halle, Jena, and Vienna. Especially T_{avg} is considerably higher in Vienna than it is in the other locations (Figure 5A). A closer inspection of the distribution of days across the 5°C intervals (Figure 5B) shows that, correspondingly, Vienna exhibits particularly high numbers of days in the intervals above 25°C, while for the colder intervals (up to 24.9°C), higher numbers of days tend to occur in Berlin, Halle, and Jena.

The annual precipitation sums in Halle, Jena, and Vienna vary significantly between their driest and wettest years (Figure 5C). In Halle, the driest year was 2022, with a PRCP of 354.7 mm, while the wettest year was 2023, reaching 590.0 mm. Jena recorded 460.6 mm in its driest year of 2022, and its wettest year was 2021, with 691.7 mm. In Vienna, the driest year also occurred in 2022, with 513.0 mm, and the wettest year was 2023, totalling 771.6 mm. In Berlin, PRCP exhibits the greatest fluctuation among all cities, ranging from 355.5 mm in 2022 to 777.4 mm in 2023. The latter amount constitutes the most abundant rainfall in a single year recorded across all sites in the sample period. However, the location with the highest precipitation altogether is Vienna, followed in descending order by Jena, Berlin, and Halle. Despite being the location with the overall most precipitation, Vienna also almost continuously possesses the most days per year without any rainfall. This suggests a clustered distribution of precipitation that is only paralleled by Berlin, where the DWP total/year occurred in 2022, and where the consecutive streaks of DWP are, on average, the longest in the data set. In the four years investigated, DWP range from 10 to 22 days in Berlin, 12 to 16 days in Halle, 10 to 16 days in Jena, and 12 to 18 days in Vienna.

RH ranges from 73.31 to 77.64% in Berlin, 72.02 to 77.05% in Halle, 69.49 to 74.34% in Jena, and is markedly lower in Vienna at 60.50 to 62.86%. VP ranges from 9.61 to 10.70 hPa and varies little between the locations and years (CV = 3.3%). These and other ranges and measures of variability of the weather parameters can be found in Table S1 in the Appendix. Overall, the parameters showing the strongest dispersion in the sample with a CV > 15% are Days <0°C, Days 0-4.9°C, Days 25-29.9°C, Days 30-34.9°C, Days > 35°C, PRCP, and DWP.

3.2.2 Normality and correlations with root traits

A Shapiro-Wilk test across all cities and years suggests normality for most weather variables except T_{avg}, Days < 0°C, RH, and VP. After a visual inspection of the measurement distributions, Days 30-34.9°C, Days > 35°C, DWP total/year, and DWP are also classified as non-normal. One-way ANOVA and Kruskal-Wallis tests

for the weather across all four years reveal significant differences between the locations only for T_{avg}, T_{max}, Days 25-29.9°C, Days 30-34,9°C, and RH, as shown in Table S1. Thus, for most parameters, the variation within a single location over the four years is equal to or greater than the variation between the sites. In particular, no significant differences were observed across locations for Days <0°C, Days 0-4,9°C, Days 5-9.9°C, Days 10-14.9°C, Days 15-19.9°C, Days 20-24.9°C, Days >35°C, PRCP, DWP total/year, DWP, and VP, indicating a strong similarity in these parameters across sites.

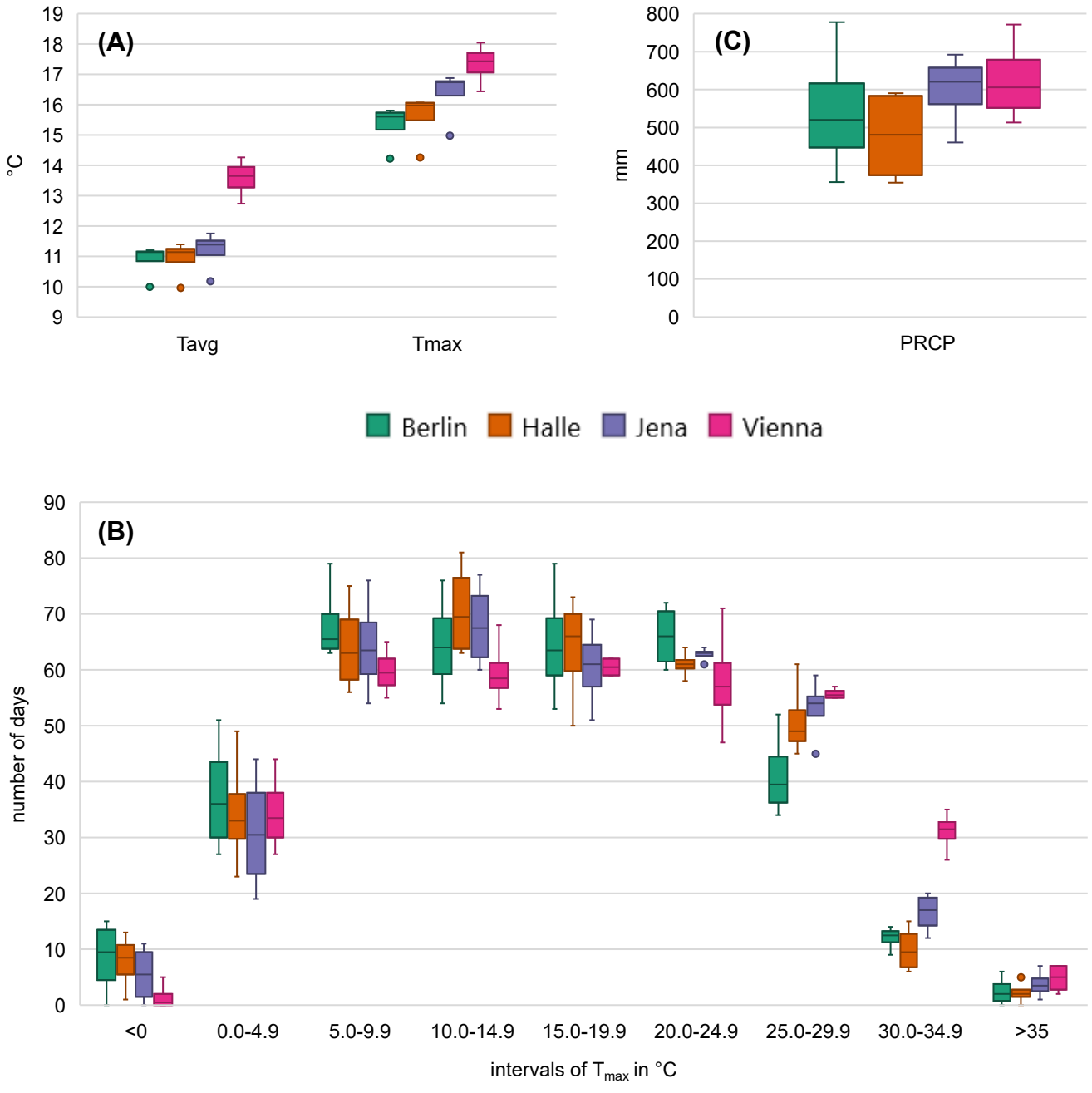

Figure 5 Differences in weather parameters between the four locations over the four-year period. **(A)** T_{avg} and T_{max}, **(B)** PRCP, **(C)** frequency of days with maximum daily temperature within a 5°C interval.

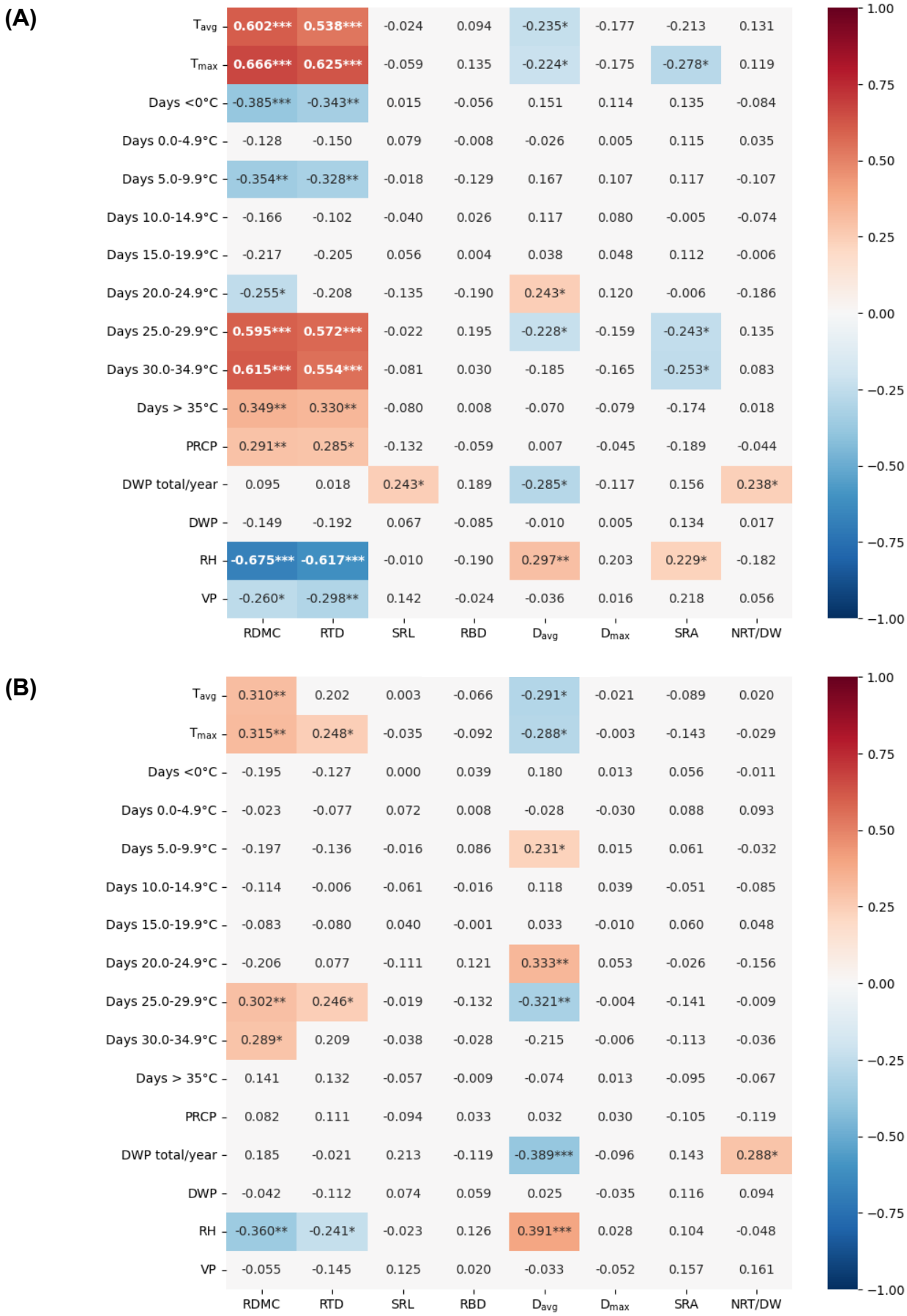

Figure 6 Spearman's ρ values for root variables with weather parameters across all locations and years. **(A)** *Salvia nemorosa* (n = 20) and **(B)** *Sanguisorba minor* (n = 19). Values reaching significance (*p < 0.05, **p < 0.01, ***p < 0.001) are coloured, with values above 0.5 or below -0.5 in bold. Negative correlations are displayed in blue, positive correlations in red. Non-significant values are included but unshaded.

Spearman's ranks for root traits with weather variables were calculated separately for the two species. Due to the small sample size and the limited location-specific differences in root and weather measurements, both times, the overall sample across all cities was used to calculate ρ values. In *Salvia nemorosa,* this produces the discernible, though not entirely continuous pattern depicted in Figure 6A. Among the root traits, RDMC and RTD show the strongest positive correlations with weather parameters, especially with T_{avg}, T_{max}, Days above 25°C, and PRCP, several of which exceed ρ = 0.5. Correspondingly, the amount of days with cooler temperatures below 24.9°C shows slightly negative correlations with RDMC and RTD, as does VP. RH is strongly negatively correlated with RDMC and RTD. Therefore, warm temperatures and high precipitation co-occur with high RDMC and RTD in *S. nemorosa,* while low temperatures, high vapor pressure, and high relative humidity coincide with lower RDMC and RTD. D_{avg} and SRA exhibit a weak pattern of correlations with weather that is reversed to that of RDMC and RTD. Overall, the weather parameters showing strong correlations with root traits roughly correspond to those differing most significantly between the locations.

Figure 6B graphically represents the same procedure applied to *Sanguisorba minor.* While the overall pattern is congruent with that of *S. nemorosa, S. minor* shows fewer significant correlations of RDMC and RTD with weather variables, which generally also reach lower ρ values. However, the relationships of D_{avg} with weather variables are slightly more pronounced in *S. minor* than in *S. nemorosa.* In both individual species, several more correlations achieve significance, though without forming a consistent pattern. Because not all root traits of *Salvia nemorosa* and *Sanguisorba minor* differ significantly between the species (see Table 4), root trait correlations with weather variables were also calculated for both species combined. The corresponding illustration can be found in Figure S1 in the Appendix. In this larger sample, the correlation patterns of RDMC and RTD with weather parameters that are also recognisable for *Salvia nemorosa* remain discernible and significant, although the ρ values decrease.

3.3 Phenology data

3.3.1 Descriptives and variability

Of the three domains under investigation, phenology is the only one with an incomplete data set. Particularly in *Sanguisorba minor,* observations are missing at the beginning and toward the end of the vegetation period due to difficulties in monitoring the first and last phenological stages of the year. Thus, for *S minor,* data coverage is below 75% for

IG, FL, SS, S50, and VP. For *Salvia nemorosa,* valid observations are slightly below 75% only for IG and VP. Data are overall most complete from the beginning of the reproductive stages to the onset of senescence. Table S2 in the Appendix contains the percentages of valid observations for each phenophase and species, along with measures of variability.

In both species, IG, FL, EF, SS, and S50 are highly dispersed, with CV values ranging from 15.5 to as high as 64.6%. Due to the variability in the timing of the end of flowering and the onset of senescence, FD and VP are also widely scattered. In contrast, FF, PF, EPF, and FFr are much more tightly grouped throughout the locations in both species (CV < 9%). This is illustrated in Figure 7, which displays the phenological development across all locations separately for the two species. The figure also shows that as the phenology advances throughout the year, the mean values of the variables, measured in days of the year, increase in a relatively steady pattern following the order of the phenophases. The difficulties in observing the first and last phenological stages of *Sanguisorba minor* are apparent in the figure as well, in a lack of data particularly for IG, FL, and S50.

3.3.2 Normality and correlations with root traits

A Shapiro-Wilk test of the phenological parameters reveals that nearly all are non-normally distributed, except for PF, S50, and VP in *S. minor*. Subsequent Kruskal-Wallis tests against the location for all non-normal variables show that significant differences occur in phenological development between the gardens (Table S2) despite the sites' close geographical proximity and similarities in weather (see Table S1). In *Salvia nemorosa,* with IG, FL, PF, EF, FFr, SS, S50, and VP, a majority of phenophases differ significantly between the sites, implying a relatively strong influence of location on phenology. In *Sanguisorba minor,* in contrast, this only applies to EPF, EF, FD, and FFr, which indicates an overall more synchronised phenological development across the sites than is the case for *S. nemorosa*. Interestingly, despite the overall low dispersion of PF in *S. nemorosa,* EPF in *S. minor,* and FFr in both species, these parameters differ significantly between the sites.

The calculation of Spearman's ρ for each root trait measurement of 2023 with the phenological observations of 2020-2022 across both species and all botanical gardens reveals a notable pattern of correlations that is depicted in Figure 8. Particularly variables concerned with the plant's reproductive phenology correlate significantly with root traits. SRL, RBD, SRA, and NRT/DW correlate negatively with FF, PF, EPF and FFr, while D_{avg} correlates positively with these phenophases. All these correlations are strong, with ρ values well above 0.7 or below -0.7 in many instances. D_{max} shows the same

correlations as D_{avg} with slightly less strength. The overall pattern also extends to the phenological stages of FL and EF, though with lower ρ values. To put simply, early occurrences particularly of reproductive phenophases correlate with higher observations

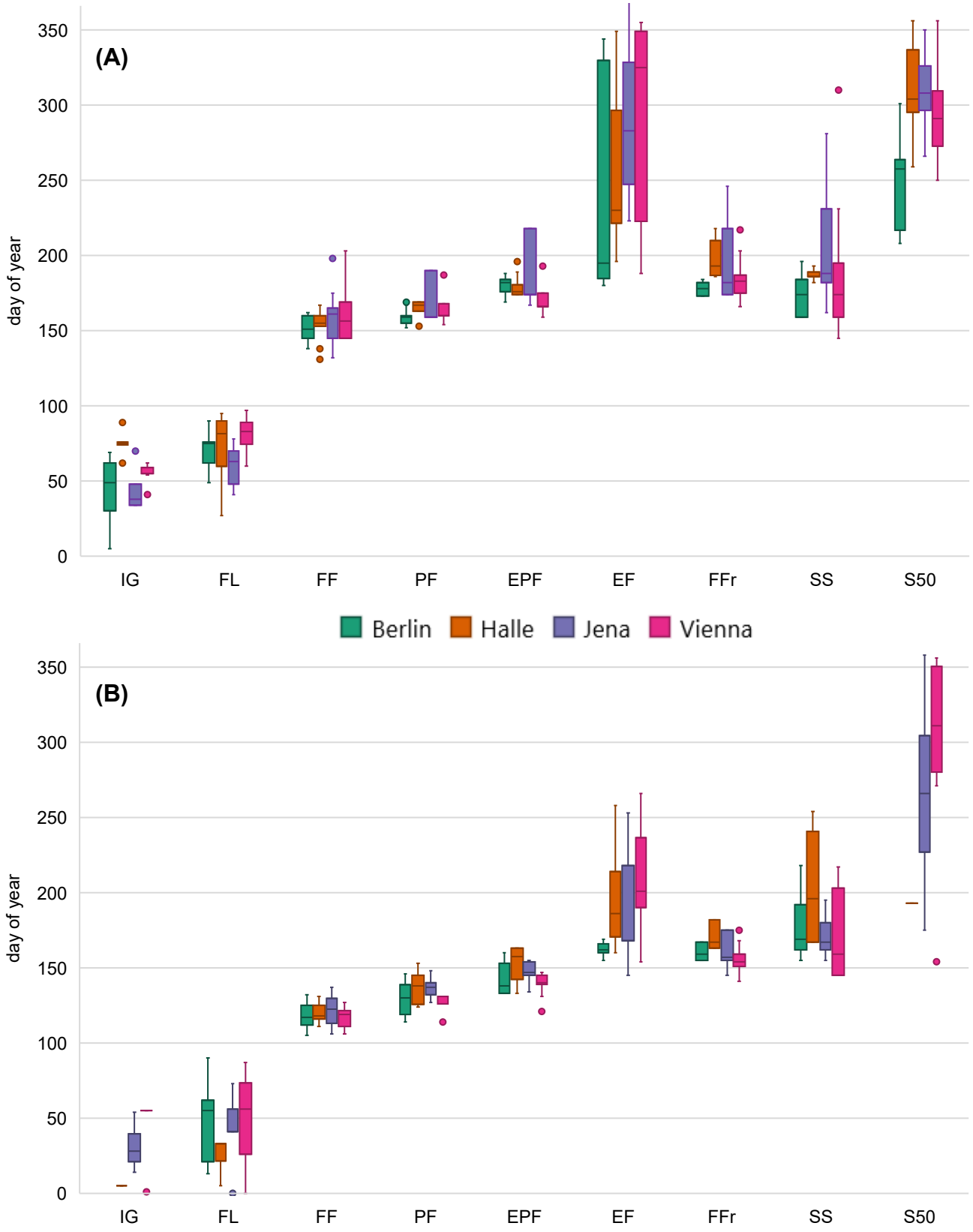

Figure 7 Phenological development of the observed plants across all locations and years. **(A)** *Salvia nemorosa,* **(B)** *Sanguisorba minor*. Data coverage for IG, FL, and S50 is low. Measurements for FF to SS are more complete (see Table S2), resulting in a more reliable depiction of their distributions.

for specific root length and area, root branching density, and the number of root tips per dry weight, but with smaller D_{avg} and D_{max}. RTD exhibits weak positive correlations with several of the phenological variables, suggesting that high root tissue densities may coincide with a slow phenological progression. RDMC, in contrast, shows very few weak correlations of inconsistent direction with weather parameters.

Spearman's ρ was also calculated individually for *Salvia nemorosa* and *Sanguisorba minor* to inspect the two species' possibly dissimilar relationship patterns of root traits with phenological development. However, as illustrated in Figure S2 in the Appendix, this procedure results in hardly any significant effects, presumably due to the very low sample sizes. The most salient correlation remaining in an individual species is that of RTD with S50 in *Salvia nemorosa,* with ρ = 0.549 (p < 0.001). For all other occurring correlations that reach significance, ρ values remain moderate to low.

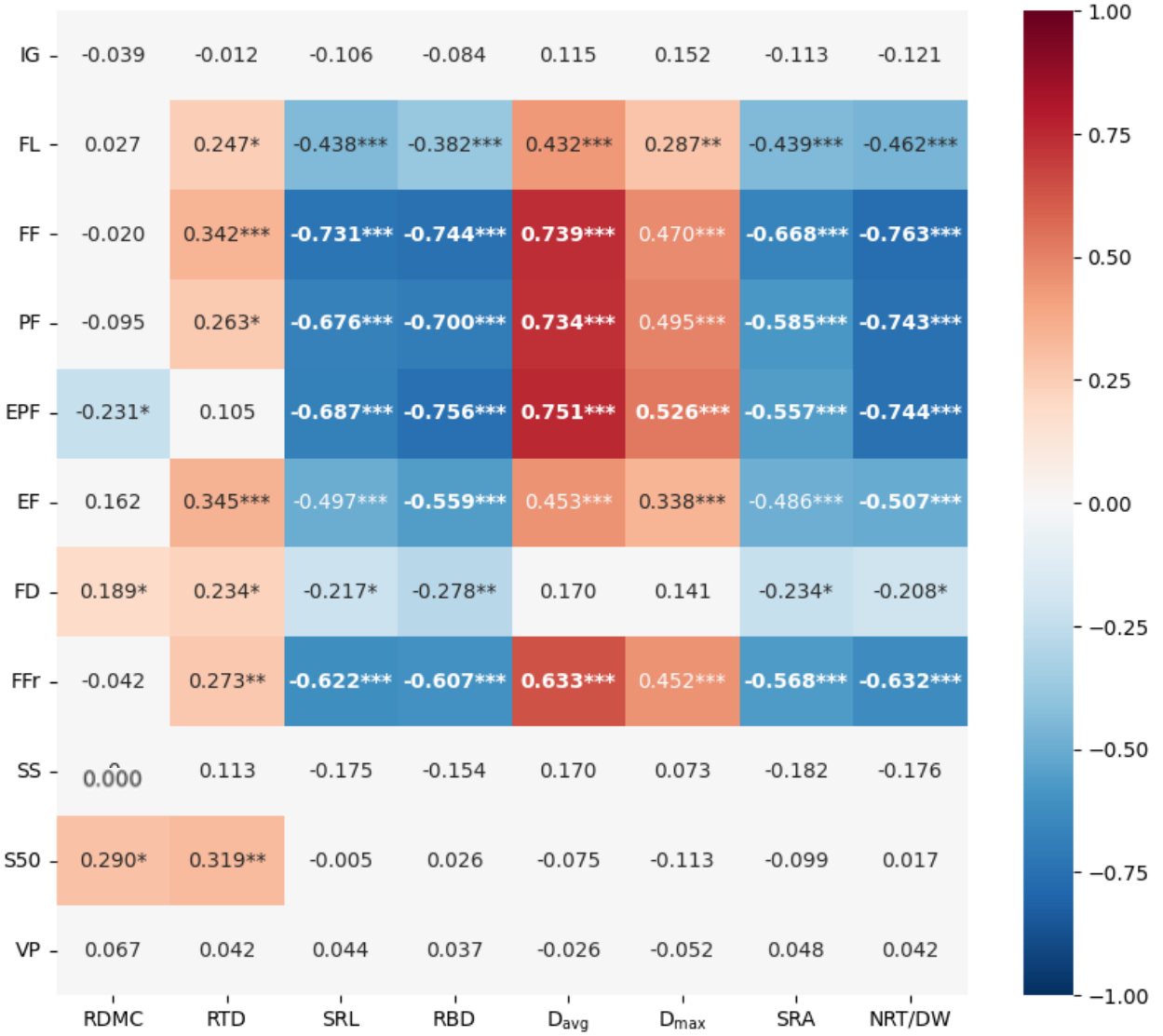

Figure 8 Spearman's ρ values for root variables with phenological variables of all four years, across both species (n = 39) and all gardens. Values reaching significance (*p < 0.05, **p < 0.01, ***p < 0.001) are coloured, with values above 0.5 or below -0.5 in bold. Negative correlations are displayed in blue, positive correlations in red. Non-significant values are included but unshaded.

4 Discussion

4.1 Key characteristics of absorptive roots

As a result of phenotypic plasticity and ontogenetic variation, plant traits can vary drastically within a species or even a single individual across time (Barton 2024: 101). Yet, a key requirement of parameters used in trait-based ecological research is to remain stable throughout a plants' ontogeny (Garbowski *et al.* 2021: 248) or as a reaction to external, environmental influences. Alternatively, if environmental factors do shape a trait, their patterns of covariation should be regular and well-known. Since roots were sampled from mature individuals only and at a single point in time for this analysis, it is impossible to determine the temporal variability of the traits surveyed here. Fortunately, many are considered to vary little with season and plant age (McCormack *et al.* 2017: 30), rendering them a suitable pool of traits to be searched for any that may be useful for trait-based investigations.

The present study cannot provide a sufficient basis for establishing universal claims regarding the regularity of traits, owing to its limited sample size and the unknown phylogeny of the sampled plants. While the explicit effort to collect genetically similar material from a single original population per species ensures comparability across the locations, it also results in a limited representation of the trait diversity possible for *Salvia nemorosa* and *Sanguisorba minor.* Values of traits observed here may vary more widely in studies utilising a more diverse genetic pool, more heterogeneous soil composition and nutrient contents, more locations, or a larger number of individuals per site. Nevertheless, strong phylogenetic signals for certain root traits have been found in such more diversified studies. These support the notion of taxon-specific stability for some traits, for example for average diameter or specific root length (Fort & Freschet 2020: 1570; Kong *et al.* 2014: 868). Thus, operating under the consideration of this investigation's limitations, some variables with comparably low dispersion within the species and across the locations can be identified from its results.

For both species scrutinised here, a narrow distribution of root branching density coincides with a lack of significant differences in this trait between the sites, indicating that RBD is developed similarly across all individuals of a species. This renders it the most stable root trait in this study, which is diametrically opposed to previous observations of high intraspecific variability in RBD. For example, it has been found to correlate with the local abundance of a species, with high competition resulting in RBD variations as an adaptive strategy (Li *et al.* 2017: 1145). Low phylogenetic conservatism found for RBD

supports a potentially powerful environmental influence on the trait (Kong *et al.* 2014: 869). However, in the present survey, RBD shows no significant correlations to weather variables for reasons that remain unclear. Experimental investigations of this trait in relation to environmental factors could contribute to a more conclusive clarification of its stability in the two species.

In the case of *Sanguisorba minor,* the average and median diameter are also grouped rather narrowly within the sample. This is plausible in view of the strong phylogenetic determination of diameters (Fort & Freschet 2020: 1573), but also somewhat surprising considering the strong ties of fine-root diameter to mycorrhizal colonisation (Bergmann *et al.* 2020: 2-3). While the latter factor does not surface in an overall strong dispersion of diameter values, it may play a role in the significant differences identified in diameters between the sites. The correlations of D_{avg} with weather variables also indicate its possible contingency on external factors. Nevertheless, despite these possible outside influences, the values for D_{avg} as well as D_{med} in *S. minor* are condensed enough to cautiously assume a low variability for these parameters across different environments. While this finding's significance is diminished by the impossibility to distinguish the diameters of order 1, 2, and 3 roots in the project at hand, as well as by the lack of information on their mycorrhizal colonisation, it still warrants further investigations into fine root diameters as a possibly stable trait of *S. minor.*

Other root traits such as specific root length, specific root area, D_{max}, or the number of root tips per dry weight exhibit high variability. SRL, for example, is known to be strongly influenced by mycorrhizal colonisation, nutrient availability, and temperature (Bergmann *et al.* 2020: 2; Fort & Freschet *et al.* 2020: 1571; Freschet, Roumet *et al.* 2021: 1125), which is reflected in the present sample in a large overall variation of SRL. In *S. minor*, SRL values are also considerably lower than those previously reported for this species, likely due to the present study's focus on fine roots, which contrasts earlier investigations' focus on entire root systems (De Lillis *et al.* 2005: 217). The stability of some root traits is further diminished by their tendency to change throughout ontogeny (Freschet, Pagès *et al.* 2021: 981). For example, SRL in the adult individuals of *Salvia nemorosa* examined here is five to ten times lower than reported for juvenile plants of *S. hispanica*. While direct comparisons between two distinct species must be approached with care, this pattern aligns with the decline in SRL from seedlings to juveniles identified in *S. hispanica* (Ianucci & Amato 2022: 3210-3211), suggesting a potential general decrease in SRL as plants mature. Similar ontogenetic changes are likely to occur in other root traits, as well.

Of all root parameters investigated, root dry matter content and root tissue density show the greatest variability and the lowest specificity. This is visible from their broad dispersion in *Sanguisorba minor*, their lower variation but significant dependence on location in *Salvia nemorosa*, and their lack of significant differences between the two species. Both RDMC and RTD also exhibit extensive correlations with weather variables, indicating that external factors may strongly influence their values. This is in accordance with a strong positive influence of atmospheric humidity on RTD found for many species, while nutrient and water availability in the soil correlate negatively with RTD (Fort & Freschet 2020: 1571). In addition, here, the dry matter content in adult *S. minor* plants is slightly higher than previously reported for younger individuals of the species, suggesting an increase in RDMC throughout ontogeny (Torres *et al.* 2022: 172-175). Overall, while RDMC and RTD certainly can and do vary between species, in this examination, their intraspecific variations across the sites are just as extensive. This encourages the assumption that RTD and RDMC might be influenced by location and environment, whereas the remaining traits that differ significantly between *S. nemorosa* and *S. minor* may depend more strongly on species.

4.2 Roots and weather

Consistent with the possibility of environmental factors influencing root tissue density and dry matter content, high temperatures co-occur with high RDMC and RTD of absorptive roots in both species. This pattern is particularly extensive in *Salvia nemorosa*, where positive correlations with RDMC and RTD can be found for average and maximum daily temperatures as well as the numbers of days above 25.0°C. At the same time, correlations with the days below 25.0°C are negative. These results correspond to previous observations of root growth and root biomass increasing with rising air or soil temperatures (Abramoff & Finzi 2015: 1056; Alsajri *et al.* 2020: 252; J. Wang *et al.* 2021: 1859; Steinaker & Wilson 2008: 1226). Therefore, wherever more days with high temperatures occur, roots may grow denser and with lower water content, or, in other words, higher carbon content. In maize plants, similarly, a positive correlation between root biomass and temperature has been observed (Vescio *et al.* 2020: 3-4), though this comparison demands careful interpretation. Since maize is a C4 monocotyledon, its root structure as well as its anatomy and metabolism differ considerably from that of the species scrutinised here. In the present setting, the abrupt reversal of signs in correlations of temperatures with RDMC and RTD at 25°C may indicate this temperature as the lower threshold for optimal growth, beyond which carbon allocation to the roots is possible with higher efficiency. The notable decrease in

effect size and significance of the correlations in *Salvia nemorosa* above 35°C might in turn point to the upper limit of its temperature optimum having been reached.

Regarding associations with parameters of moisture, RDMC and RTD correlate positively with the yearly precipitation sum, which is contradictory to findings of root biomass allocation increasing when precipitation decreases (Sun *et al.* 2022: 7; Xing *et al.* 2024: 7). Corresponding effects of higher root densities during droughts, indicating an increased carbon allocation to the roots, have indeed been observed before in *Sanguisorba minor* (De Lillis *et al.* 2005: 220) but do not surface here. Instead, this analysis' findings are consistent with contrasting observations of root biomass increasing and decreasing with rising and falling precipitation, respectively (Calleja-Cabrera *et al.* 2020: 12; Slette *et al.* 2023: 5; P. Wang *et al.* 2020: 1496; Xing *et al.* 2024: 4). RDMC and RTD decline with high relative humidity and, in *Salvia nemorosa,* with vapor pressure, suggesting that high RH and VP, too, coincide with inhibited biomass allocation to the roots. This contrasts the positive effect of humidity on RTD found by Fort and Freschet (2020: 1571). To resolve these contradictory results and to understand the impact of RH and VP on root form, a more in-depth examination is needed into the (likely transpirational) mechanisms by which moisture-related atmospheric parameters influence root morphology.

While in this study, as in others before, the mere precipitation sum shows no significant correlations with any root traits (Abramoff & Finzi 2015: 1056; Addo-Danso *et al.* 2020: 6; McCormack *et al.* 2020: 1829; Slette *et al.* 2023: 7), the yearly number of days without precipitation does. A high total number of DWP per year correlates positively with the number of root tips per dry weight, and negatively with average diameters. In *Salvia nemorosa,* it also correlates positively with specific root length. Thus, a high number of dry days, implying precipitation to be low or irregularly distributed across the season, correlates with the formation of long, thin roots with many root tips. A similar pattern has been observed with irregular precipitation correlating positively to root length but negatively to diameters (McCormack *et al.* 2020: 1829). These findings coincide with a hypothesised mechanism of reaction to drought for herbaceous plants, whereby, under conditions inhibiting carbon allocation to the roots, the plants opt for the formation of highly acquisitive structures to maximise their water and nutrient uptake per unit of biomass (P. Wang *et al.* 2020: 1496). In support of this, low precipitation and soil moisture have been found to induce longitudinal growth in roots, possibly to reach deeper soils where drought can be avoided (Karlova *et al.* 2021: 1058; Li *et al.* 2017: 1144; Steinaker & Wilson 2008: 1226-1227). Accordingly, in this investigation, the

correlations of low precipitation with low RDMC and RTD, as well as the weak positive correlation between a high number of dry days per year with long, thin, richly tipped roots, suggest a tendency of acquisitive root formation at low precipitation conditions. Though belowground responses to drought stress can vary widely between species (Larson & Funk 2016: 834), *Salvia nemorosa* and *Sanguisorba minor* both exhibit this pattern. In contrast to the total number of DWP/year showing these effects, the longest streak of DWP is not significantly correlated to any of the root traits.

Interestingly, while Fort and Freschet (2020: 1570) speculate that specific root length would be more strongly influenced by weather than diameters, the opposite can be observed here, with D_{avg} showing stronger correlations with weather than SRL. Indeed, the latter seems entirely unaffected by temperature. In general, the present results do not show the strong relationships of SRL or RBD with temperature that have been observed before. The most likely reason for such contradictory findings of positive (Addo-Danso *et al.* 2020: 8-9; Karlova *et al.* 2021: 1062), negative (Fort & Freschet 2020: 1566; S. Wang *et al.* 2021: 7), and non-existent relationships between temperature and root length (J. Wang *et al.* 2021: 1859) is that, according to the specific environments and stresses plants are exposed to, and particularly as a response to cold stress, root morphological reactions can vary strongly (Karlova *et al.* 2021: 1063; see also Addo-Danso *et al.* 2020: 9; Luo *et al.* 2020: 12). Since the temperature likely was below the optimum for root growth in *Salvia nemorosa* and *Sanguisorba minor* during large stretches of the observation period, this may account for the current findings' lack of correspondence with previous results.

Specific root area in *S. nemorosa* and average diameters of both species show a negative correlation with T_{avg} and T_{max}. Maximum diameters, in contrast, are unrelated to temperature. Overall, SRA and D_{avg} exhibit a relationship pattern with weather parameters that is roughly reversed to that of RTD and RDMC. High temperatures thus co-occur with the formation of thin, dense roots that are commonly associated with high nutrient absorption capacity and long tissue life (Freschet, Pagès *et al.* 2021: 1025, 1027; Klimešová & Herben 2023: 5). Vice versa, low temperatures coincide with the occurrence of thicker roots with lower dry matter concentrations. This pattern of correlations observed here contrasts with established findings of larger diameters being the result of higher temperatures (Calleja-Cabrera *et al.* 2020: 6; S. Wang *et al.* 2021: 7). According to the fast versus slow dimension of the root economics spectrum, large diameters are associated with resource conservation, while acquisitive strategies coincide with longer, thinner, densely branched roots (Addo-Danso *et al.* 2020: 9;

Bergmann *et al.* 2020: 2; Freschet, Roumet *et al.* 2021: 1125, 1145; McCormack *et al.* 2017: 32). The negative correlation of temperatures with diameter found here thus points towards resource conservation strategies at lower temperatures, which should result in amplified carbon storage in the roots. However, the low RDMC and RTD coinciding with low temperatures do not fit this assumed mechanism (Freschet, Pagès *et al.* 2021: 1027) and instead hint at difficulties with transporting carbon to the roots in cold conditions, prompting a possible alternative scenario.

Low root tissue density and dry matter content with simultaneously large diameters could be indicative of a strategy to maximise specific root area with as little carbon investment as possible. Apart from increasing SRL, other mechanisms employed by plants to mitigate problematic conditions include intensifying biomass allocation to the fine roots, increasing rooting depth, or utilising mycorrhizal symbiosis (McCormack *et al.* 2020: 1831; Weemstra, Valverde-Barrantes *et al.* 2023: 3). Here, we may see yet another strategy for improving substance uptake at the root surface. Along with increasing fine-root diameters, SRA also rises, circumventing the need for high SRL to produce a large root-soil interface. In this scenario, large diameters would be indicative of resource acquisition rather than conservation. Additionally, high D_{avg} of fine roots occurring at the same conditions as low RDMC and RTD point toward a large SRA achieved via minimal carbon investments. This may be an economic strategy to maximise SRA for efficient nutrient uptake that is adapted to the specific stresses the plants in this study were exposed to, particularly that of temperatures ranging below the optimum for root growth. It may also indicate strong mycorrhizal involvement which is associated with high D_{avg} and low SRL (Bergmann *et al.* 2020: 2; Weemstra, Valverde-Barrantes *et al.* 2023: 4). The largely absent direct correlations between SRA and weather parameters in both species do not contradict this hypothetical mechanism. Plants' varied investment in root diameters, length, and tissue densities affects how SRA is generated in relation to the amount of carbon allocated to the roots, but it does not necessarily determine the total amount of SRA attained.

However, this mechanism of SRA increase via diameter expansion at adverse conditions is highly speculative, and experimental data on the responses of root traits to cold stress in *Salvia nemorosa* and *Sanguisorba minor* are indispensable to confirm or refute it. It is also important to note that the correlations giving rise to this hypothesis are weak, and upon inspecting the relationships of root traits with weather parameters for both species together, almost all correlations disappear (Figure S1). Only those of RDMC and RTD with various weather variables remain, as well as very weak correlations

of D_{avg} with parameters of moisture. Thus, while RDMC and RTD seem genuinely affected by weather variables in the surveyed species, other morphological and architectural traits may turn out to be independent in a larger sample.

4.3 Roots and phenology

The relationships between roots and phenology are more distinct and continuous than those of roots with weather parameters. While hardly any correlations occur between root traits and aboveground phenology for the separate species (Figure S2), likely due to the small sample sizes, a striking pattern emerges when the two species are combined to increase the sample size. The most salient relationships of root traits and phenology occur with phenophases from the first flower to the end of peak flowering, and with the first ripe fruit. Specific root length, area, and measures of root branching consistently exhibit very strong negative correlations with these phenological stages. At the same time, D_{avg}, and with slightly lower intensity also D_{max}, correlate strongly positively with the phenological progression. This indicates that an early attainment of reproductive phenophases in *Salvia nemorosa* and *Sanguisorba minor* coincides with low root diameters, but also with high SRL, SRA, RBD, and NRT/DW. Such a pattern is typical of roots adapted for resource acquisition and the efficient use of localised soil nutrients (Freschet, Pagès *et al.* 2021: 1022, 1025; Freschet, Roumet *et al.* 2021: 1125). In the present study, this acquisitive root trait syndrome occurs much more consistently in connection with phenology than with the weather.

Roots with high SRL, SRA, and volume are assumed to accelerate vegetative growth phases, particularly in the presence of growth-promoting rhizobacteria (Lyu *et al.* 2022: 5). In such cases, to enhance acquisitive root formation, plants might prioritise resource allocation to the roots. This shift in resource partitioning could manifest in measurement values as competition between root and shoot growth. For instance, the negative correlations observed in *Sanguisorba minor* and *Salvia hispanica* between tap root SRL and aboveground traits – such as plant height, total leaf area, shoot biomass, and shoot dry weight (Ianucci & Amato 2021: 3212; Torres *et al.* 2022: 174-178) – suggest strong competition for photosynthates between the aerial and subterranean parts of the plants. Such competition likely results in slowed or reduced vegetative growth, favouring root development instead. However, as Sporbert *et al.* (2022: 2205) have observed, smaller aboveground phenotypes tend to flower and fruit earlier than larger plants. Thus, a prioritisation of root over shoot growth, as reported for *S. minor* and *S. hispanica*, may result in smaller plants with accelerated reproduction. The extensive

and strong positive correlations between acquisitive root traits and fast phenological progression found here support this hypothesis.

The pattern of early reproductive phenological stages co-occurring with long, thin, highly branched and richly tipped absorptive roots extends with somewhat weaker correlations also to FL and EF, while remaining most consistent across flowering and fruiting. During these reproductive phenophases, changes in the correlations between root and shoot growth could be expected due to the carbon competition the formation of fruits and seeds represents for root growth (Calleja-Cabrera *et al.* 2020: 7; Radville *et al.* 2016: 3623). Correspondingly, a sink effect during flowering and fruiting has been observed for example in rapeseed, where larger root systems correlate with later flowering times (Fletcher *et al.* 2015: 248). In the present data, however, the occurrence of a strongly branched root system with high SRL and SRA coincides with early reproduction. Torrion *et al.* (2012: 1707) have found a similarly unimpeded progression of root development throughout the reproductive stages in soybeans, providing another example of absent negative interactions between reproduction and root growth. This phenomenon may be related to a plant's overall vegetation period length. Plants with short aboveground growth periods tend to separate root and shoot growth temporally, whereas longer vegetation periods are accompanied by larger overlaps in root and shoot development (Palacio & Montserrat-Martí 2007: 529). As for both *Salvia nemorosa* and *Sanguisorba minor* fairly long vegetation periods were observed, these species may be able to maintain simultaneous root and shoot growth. In this case, carbon investments into the roots resulting in an increased SRA would facilitate nutrient uptake and in turn enable a faster aboveground carbon gain (Weemstra, Kuyper *et al.* 2023: 8). Thus, if *S. nemorosa* and *S. minor* are indeed capable of continuing root growth throughout the entire vegetation period, this may explain their continued correlations of roots with phenology across the reproductive stages.

In summary, the strategy described above entails a cooccurrence of highly acquisitive roots with the root-shoot competition and small vegetative phenotypes reported by Ianucci and Amato (2021: 3212) and Torres *et al.* (2022: 174-178). At the same time, it leads to earlier reproduction (Sporbert *et al.* 2022: 2205). In plants with long vegetation periods, as observed in this thesis, the overlapping root and shoot growth found by Palacio and Montserrat-Martí (2007: 529), along with their mutual advancement according to Weemstra, Kuyper *et al.* (2023: 8), minimises negative interactions between roots and shoots. This dynamic produces a pattern of root competition with shoot size during vegetative stages, while also resulting in the strong correlations found here

between acquisitive roots and rapid phenological progression during generative phases. For an assessment of whether this leads to an overall increase of reproductive success, additional data is required, for example on seed numbers and weights or germination rates.

The duration of flowering has fewer clear relationships with root traits. Significant negative correlations do occur for FD with SRL, SRA, RBD, and NRT/DW. This indicates that a short flowering duration, or rapid flowering progression, again correlates weakly with the presence of long and highly branched roots. However, FD is not significantly related to average or maximum root diameters. Absorptive root diameters thus covary with the point in time where stages of flowering occur, but not with their duration. This deviation from the overall correlation patterns exhibited by the other root traits could indicate additional influences specifically on root diameters, such as mycorrhizal colonisation (Bergmann *et al.* 2020: 3; Klimešová & Herben 2023: 8). Unfortunately, since this factor could not be included in this survey, its role in determining the root diameters recorded here cannot be clarified.

Interestingly, the correlations of roots with shoot phenology largely disappear wherever the phenological progression is not immediately tied to reproduction. Neither initial growth nor start of senescence or vegetation period length correlate significantly with any root trait. While this may well be due to low data quality owing to difficulties in observing the corresponding phenophases, it could also signify a stronger link between phenology and root makeup within than outside of the reproductive phase. Since root growth is assumed to continue or even increase in autumn while aboveground plant organs age (Palacio & Montserrat-Martí 2007: 529; Radville *et al.* 2016: 3623-3624), these results may be indicative of a separation of plant organ development once reproduction is completed.

RTD shows low, but consistently positive correlations with FL, FF, PF, EF FD, FFr, and S50, revealing that root tissue density is higher if these phenological stages are reached later in the year, or when the flowering stages last longer. Accordingly, high RTD in short, thick roots has been found to be associated with slow turnover rates and long root lifespans, while longer, thinner roots with low RTD possess a shorter lifespan. The former occur more frequently in perennial species, while the latter are more likely to occur in short-lived, annual species (Klimešová & Herben 2023: 5-6). In this regard, it is quite conceivable that fast phenological progression is associated with longer, thinner roots, as suggested by the findings of this study. In an extension of the fast-slow continuum of root

traits described by Bergmann *et al.* (2020: 2), the parameters associated with shorter root lifespans can be seen here to also coincide with a fast progression through aboveground phenological stages. These results also suggest some synchronisation of belowground carbon allocation and aboveground development speed, which, as trivial as it may appear, is not self-evident given the instances of delays and root-shoot trade-offs identified before (Abramoff & Finzi 2015: 1058; Ianucci & Amato 2021: 3212; Steinaker & Wilson 2008: 1224; Torres *et al.* 2022: 174).

Similarly to RTD, root dry matter content also correlates weakly positively to FD and S50. However, it shows a negative correlation with EPF. Thus, while long flowering duration and late 50% senescence are associated with high RDMC, a late end of peak flowering correlates with low RDMC, making it the only root trait exhibiting inconsistent relationships with the phenological progression. The associations found here between RDMC and senescence align with the findings of Ye *et al.,* who revealed in their trait network analysis that low RDMC correlates with high leaf senescence (2024: 7). Together, this strongly suggests some importance of high RDMC as a predictor of delayed aboveground senescence. Overall, in agreement with known associations of high RTD and RDMC with conservative growth strategies and stress resistance (Bergmann *et al.* 2020: 2; Freschet, Pagès *et al.* 2021: 1027), high root tissue density and root dry matter content are clearly accompanied by slow phenological progression and ageing. In conclusion, this link between root tissue properties and aboveground development, along with the diverse relationships between root morphology and phenology outlined in this thesis, provide novel insights into the coordination of plant organs both above and below ground.

4.4 Limitations, strengths, and future directions

Some of this survey's shortcomings stem from organisational challenges typical for studies conducted across multiple locations and large teams. Despite best efforts to ensure comparability between the mix beds, small topographical variations in slope and exposition (Primka *et al.* 2021: 15), as well as slight differences in soil composition, nutrient contents, or the frequency of gardening interventions taken to protect plants from dying may have impacted root growth and form. Another potential limitation lies in the decision not to record switches to new plants during phenological monitoring when the original plants had died, nor to document whether the newly selected plants were of vegetative or generative origin. For phenological observations this is inconsequential, but since vegetatively reproduced individuals differ in their fine root structure and

development from generatively reproduced plants (Freschet, Pagès *et al.* 2021: 1004; Klimešová & Herben 2023: 5; Klimešová *et al.* 2018: 2119), this may have affected the roots harvested. However, the insights that can be gained from the data collected in this multi-garden project easily outweigh these problems.

Other issues, such as varying degrees of degradation and difficulties in harvesting homogeneous material across the plants, come naturally with observing belowground plant organs. In some cases, only very little root material or superficial roots could be salvaged. Especially in the case of *Salvia nemorosa*, the main tap root was often difficult to recover fully, as it extended deeper into the soil than could be excavated manually. In such cases, where significant portions of the root likely remained in the soil, only the more accessible, superficial parts of the root system could be harvested for analysis. As a result, the rather arbitrarily chosen root sections from which material was collected can hardly be considered representative for the whole plant's root system (McCormack 2017: 29). Given these factors, the results and insights presented in Sections 3 and 4 should be interpreted with caution and considered as indicative rather than definitive.

Additionally, the correlations found here do not depict the whole spectrum of possible links between root growth and external factors, and they must not be mistaken for causal relationships. Of the aspects not included in this study, it is particularly unfortunate that firstly, the colonisation of roots with mycorrhizal fungi could not be assessed despite its well-known, strong influence on root morphology (Bergmann *et al.* 2020: 5; McCormack *et al.* 2015: 513-514). Secondly, root traits were selected for their suitability for efficient measurement rather than, as strongly advocated by Freschet and Roumet *et al.* (2021: 1124), based on their common connections to specific ecological functions. This severely limits the power of illustrating the traits' impacts on specific aspects of plant functioning. Further, the choice of traits measured here does not permit any conclusions regarding the association between various dimensions of root traits, such as anatomy, morphology, physiology, or chemistry, as recommended by McCormack *et al.* (2017: 33), because only morphological and some architectural traits were measured. Finally, it was not possible to investigate the sample plants' phylogeny. Numerous such factors possibly influencing roots' form and their relationship to aerial plant organs could not be accounted for in the present thesis.

Still, despite these limitations, and despite the sample size per garden being too small for a site-specific view into the correlations, considerable relationships occur between several root traits and weather parameters in the overall sample. For the relationship between roots and shoot phenology, the strong correlations found even form a distinctive pattern. This thesis represents a rare effort to scrutinise such relationships between form-related root traits and plants' phenological progression above the ground. It is also one of the few analyses to integrate the somewhat understudied dimension of architectural root traits with the historically more frequently examined morphological traits (Langguth *et al.* 2023: 1427) in an attempt to enhance understanding of this domain. The clear associations especially for the relationship of root form with shoot phenology that are identified here certainly warrant a closer inspection on a finer time scale. Experimental and long-term investigations into the association of root morphology and shoot phenology are needed to determine their temporal and causal interaction. These relationships between acquisitive root formation and phenological progression are vital for understanding how plants functionally adapt to their environment.

Another main insight from this study, particularly from its limitations, is the necessity for larger sample sizes and more complete phenological observations. Such more comprehensive data sets are vital for a clearer determination of whether the correlations between root, shoot, and weather parameters genuinely originate from physiological processes or merely present statistical artefacts. The need for better data unfortunately applies especially to those phenophases that are the most difficult to observe. However, careful efforts to collect high-quality experimental and observational information over longer periods of time, as is currently undertaken for example by PhenObs (iDiv 2024), will aid the resolution of the questions raised in this thesis.

On a very fundamental level, the stability of RBD and D_{avg} found here prompts the question whether this pattern persists across ontogeny both within individual plants and between species. The singular instance of observing low variability in these traits in the small sample at hand does not provide a solid foundation for drawing meaningful or reliable conclusions. Further investigations may clarify whether RBD could indeed be stable within a species across individual development and different environments. Additional research is also necessary to determine how consistently D_{avg} presents a narrowly distributed trait in *S. minor.* In view of this species' significant dependence of D_{avg} on location, as well as the correlation patterns of both species' D_{avg} and D_{max} with phenology differing slightly from those of other root traits, more detailed insights are needed into what influences root diameters and how this may affect aerial plant organs.

Regarding roots' dependence on weather, the impact of VP and particularly of RH on root traits requires clarification. Atmospheric humidity is most directly connected to transpirational processes in the leaf, which are in turn influenced by vegetative makeup, transport capacities, and ultimately the water and nutrient uptake mechanisms of the plant. This axis thus intimately connects the three domains of roots, shoots, and atmosphere, which is indicated also by the significant correlations found in this thesis. The specific mechanisms whereby these influences are transmitted through the plant, however, still need to be explained. Another question connected to weather parameters arises regarding the increase of absorptive root diameter at low carbon costs. This finding may indicate a strategy for improved soil exchange that is employed by plants under conditions of water and low-temperature stress. Whether this phenomenon truly represents an alternative mechanism to the much more commonly observed SRL increase must be confirmed through experimental studies before its significance can be confidently assessed.

The correlations of root form with phenology identified here constitute novel insights that encourage a more detailed examination of the interactions between above- and belowground plant organs. Exploring how root form and phenology covary, and what this might reveal about plant functioning, are promising avenues for further research. Elucidating systematic associations between these domains will provide valuable insights into the role of roots in ecosystem functioning, which is particularly relevant in view of the currently shifting environmental conditions. To facilitate research efforts, and to make root data that is so labour-intensive to obtain more easily accessible to the scientific community, contributing to databases such as GRooT (Guerrero-Ramírez *et al.* 2021) or FRED (Iversen *et al.* 2021) is essential. Expanding these data collections to include phenological information has the potential to further enhance their utility for future studies.

5 Conclusion

To address the scarcity of plant root data and the limited understanding of how external factors influence root form, this thesis aimed to explore the relationships of root morphology and architecture with weather and with the development of aboveground plant organs. The coordinated multi-garden sampling conducted for *Salvia nemorosa* and *Sanguisorba minor* allowed for a detailed analysis and comparison of several root traits across the four study sites. The data generated from the collected root material reveals high intra- and interspecific variability for most traits, but only few of these measurements differ significantly between the gardens. Among the root traits examined, only root branching density and, to some extent, average diameter demonstrated high stability across individuals both within and between the locations.

Contradictions seem to be a common occurrence in root research, which may explain why the correlations observed in this thesis align with some previous findings but conflict with others. Here, low precipitation coincides with the formation of long, thin, densely branched roots, which fits mechanisms of acquisitive root formation under adverse conditions that have been observed before (Li *et al.* 2017: 1144; McCormack *et al.* 2020: 1828; P. Wang *et al.* 2020: 1496). Based on my findings of low root tissue densities and dry matter concentrations coinciding with large root diameters, I also postulate a possible alternative strategy for a similar increase of uptake efficiency under unfavourable conditions. When carbon allocation to the roots is inhibited, plants may expand their fine-root diameters instead of enhancing longitudinal growth and branching, in order to maximise root-soil interactive surface for substance uptake.

To the best of my knowledge, this thesis contains one of the first attempts to scrutinise the relationships of root form with shoot phenology. It very clearly shows that root forms commonly associated with acquisitive functions are strongly correlated to a fast shoot development, suggesting plants' belowground strategy to also be reflected in organs above the ground. Simultaneously, root observations associated with conservative, slow strategies co-occur with a decelerated phenological development. These findings extend the slow-fast root trait continuum defined by Bergmann *et al.* (2020: 2) to shoots, and they suggest a stronger coordination between above- and belowground traits than described before.

Overall, this thesis contributes to a growing body of knowledge on morphological and structural root traits, as well as their interactions with weather and aboveground organs. It highlights interesting avenues for further research that will be crucial for

developing a better understanding of how root traits relate to overall plant functioning (Freschet, Roumet *et al.* 2021: 1151). A deeper knowledge of roots' interrelations with climatic influences and shoot growth is essential for understanding and predicting the covariation between these domains and its implications for various ecosystems. Thus, root traits' reactions to external influences and their ensuing effects on plant functioning entail profound implications for conservation efforts and agricultural practices in the face of climate change.

References

Abramoff, R. Z., & Finzi, A. C. (2015). Are above- and below-ground phenology in sync? *New Phytologist 205*(3), 1054-1061. https://doi.org/10.1111/nph.13111

Addo-Danso, S. D., Defrenne, C. E., McCormack, M. L., Ostonen, I., Addo-Danso, A., Foli, E. G., Borden, K. A., Isaac, M. E., & Prescott, C. E. (2020). Fine-root morphological trait variation in tropical forest ecosystems: An evidence synthesis. *Plant Ecology 221*(1), 1-13. https://doi.org/10.1007/s11258-019-00986-1

Alsajri, F. A., Wijewardana, C., Rosselot, R., Singh, B., Krutz, L. J., Gao, W., & Reddy, K. R. (2020). Temperature effects on soybean seedling shoot and root growth and developmental dynamics. *Journal of the Mississippi Academy of Science 65*(3), 247-257.

Alvarez-Uria, P., & Körner, C. (2007). Low temperature limits of root growth in deciduous and evergreen temperate tree species. *Functional Ecology 21*(2), 211-218. https://doi.org/10.1111/j.1365-2435.2007.01231.x

Barton, K. E. (2024). The ontogenetic dimension of plant functional ecology. *Functional Ecology 38*(1), 98-113. https://www.doi.org/10.1111/1365-2435.14464

Bergmann, J., Weigelt, A., van Der Plas, F., Laughlin, D. C., Kuyper, T. W., Guerrero-Ramirez, N. R., Valverde-Barrantes, O. J., Bruelheide, H., Freschet, G. T., Iversen, C. M., Kattge, J., McCormack, M. L., Meier, I. C., Rillig, M. C., Roumet, C., Semchenko, M., Sweeney, C. J., van Ruijven, J., York, L. M., & Mommer, L. (2020). The fungal collaboration gradient dominates the root economics space in plants. *Science Advances 6*(27), Article 3756. https://doi.org/10.1126/sciadv.aba3756

Burnett, S. E., Pennisi, S. V., Thomas, P. A., & van Iersel, M. W. (2005). Controlled drought affects morphology and anatomy of *Salvia splendens*. *Journal of the American Society for Horticultural Science 130*(5), 775-781. https://doi.org/10.21273/JASHS.130.5.775

Calleja-Cabrera, J., Boter, M., Oñate-Sánchez, L., & Pernas, M. (2020). Root growth adaptation to climate change in crops. *Frontiers in Plant Science 11*, Article 544. https://doi.org/0.3389/fpls.2020.00544

Christodolou, M. D., Clark, J. Y., & Culham, A. (2020). The Cinderella discipline: Morphometrics and their use in botanical classification. *Botanical Journal of the Linnean Society 194*(4), 385-396. https://doi.org/10.1093/botlinnean/boaa055

De Lillis, M., Manes, F., & Tufaro, F. (2005). Root plasticity in Mediterranean herbaceous species. *Plant Biosystems 139*(2), 214-221. https://doi.org/10.1080/11263500500160542

Du, Q., Jiao, X., Song, X., Zhang, J., Bai, P., Ding, J., & Li, J. (2020). The response of water dynamics to long-term high vapor pressure deficit is mediated by anatomical adaptations in plants. *Frontiers in Plant Science 11,* Article 758. https://doi.org/10.3389/fpls.2020.00758

DWD Climate Data Center. (2024). *Daily station observations precipitation height in mm, Daily station observations of mean temperature at 2 m above ground in °C, Daily station observations of maximal temperature at 2 m above ground in °C, Daily mean of relative humidity at 2 m above ground in %, Daily station observations of mean vapor pressure in hPa.* Deutscher Wetterdienst, Bundesministerium für Digitales und Verkehr. Retrieved April 20, 2024, from https://cdc.dwd.de/portal/202209231028/

Ficken, C. D., & Wright, J. P. (2019). Nitrogen uptake and biomass resprouting show contrasting relationships with resource acquisitive and conservative plant traits. *Journal of Vegetation Science 30*(1), 65-74. https://doi.org/10.1111/jvs.12705

Fischer, M. A., Oswald, K., & Adler, W. (2008). *Exkursionsflora für Österreich, Liechtenstein und Südtirol* [Excursion flora for Austria, Liechtenstein, and South Tyrol] (3rd ed.). Land Oberösterreich.

Fitter, A. H. (1982). Morphometric analysis of root systems: Application of the technique and influence of soil fertility on root system development in two herbaceous species. *Plant, Cell and Environment 5*(1), 313-322.

Fletcher, R. S., Mullen, J. L., Heiliger, A., & McKay, J. K. (2015). QTL analysis of root morphology, flowering time, and yield reveals trade-offs in response to drought in *Brassica napus*. *Journal of Experimental Botany 66*(1), 245-256. https://doi.org/10.1093/jxb/eru423

Fort, F., & Freschet, G. T. (2020). Plant ecological indicator values as predictors of fine-root trait variations. *Journal of Ecology 108*(4), 1565-1577. https://doi.org/10.1111/1365-2745.13368

Freschet, G. T., Pagès, L., Iversen, C. M., Comas, L. H., Rewald, B., Roumet, C., Klimešová, J., Zadworny, M., Poorter, H., Postma, J. A., Adams, T. S., Bagniewska-Zadworna, A., Bengough, A. G., Blancaflor, E. B., Brunner, I., Cornelissen, J. H. C., Garner, E., Gessler, A., Hobbie, S. E., Meier, I. C., Mommer, L., Picon-Cochard, C., Rose, L., Ryser, P., Scherer-Lorenzen, M., Soudzilovskaia, N. A., Stokes, A., Sun, T., Valverde-Barrantes, O. J., Weemstra, M., Weigelt, A., Wurzburger, N., York, L. M., Batterman, S. A., de Moraes, N. G., Janeček, Š., Lambers, H., Salmon, V., Tharayil, N., & McCormack, L. (2021). A starting guide to root ecology: Strengthening ecological concepts and standardising root classification, sampling, processing and trait measurements. *New Phytologist 232*(3): 973-1122. https://doi.org/10.1111/nph.17572

Freschet, G. T., Roumet, C., Comas, L. H., Weemstra, M., Bengough, A. G., Rewald, B., Bardgett, R. D., De Deyn, G. B., Johnson, D., Klimešová, J., Lukac, M., McCormack, M. L., Meier, I. C., Pagès, L., Poorter, H., Prieto, I., Wurzburger, N., Zadworny, M., Bagniewska-Zadworna, A., Blancaflor, E. B., Brunner, I., Gessler, A., Hobbie, S. E., Iversen, C. M., Mommer, L., Picon-Cochard, C., Postma, J. A., Rose, L., Ryser, P., Scherer-Lorenzen, M., Soudzilovskaia, N. A. Sun, T., Valverde-Barrantes, O. J., Weigelt, A., York, L. M., & Stokes, A. (2021). Root traits as drivers of plant and ecosystem functioning: Current understanding, pitfalls and future research needs. *New Phytologist 232*(3), 1123-1158. https://doi.org/10.1111/nph.17072

GeoSphere Austria data hub (2024). *Stationsdaten: Messstationen Tagesdaten v2* [Station data: Daily measuring station data v2]. GeoSphere Austria – Bundesanstalt für Geologie, Geophysik, Klimatologie und Meteorologie. Retrieved April 20, 2024, from https://data.hub.geosphere.at/group/stationsdaten

Garbowski, M., Johnston, D. B., & Brown, C. S. (2021). Leaf and root traits, but not relationships among traits, vary with ontogeny in seedlings. *Plant and Soil 460*(1-2): 247-261. https://doi.org/10.1007/s11104-020-04790-z

German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig. (2024). *PhenObs.* https://www.idiv.de/de/web/phenobs.html

Guerrero-Ramirez, N., Mommer, L., Freschet, G. T., Iversen, C. M., McCormack, M. L., Kattge, J., Poorter, H., van der Plas, F., Bergmann, J., Kuyper, T. W., York, L. M., Bruelheide, H., Laughlin, D. C., Meier, I. C., Roumet, C., Semchenko, M., Sweeney, C. J., van Ruijven, J., Valverde-Barrantes, O. J., Aubin, I., Catford, J. A., Manning, P., Martin, A., Milla, R., Minden, V., Pausas, J. G., Smith, S. W., Soudzilovskaia, N. A., Ammer, C., Butterfield, B., Craine, J., Cornelissen, J. H. C., de Vries, F. T., Isaac, M. E., Kramer, K., König, C., Lamb, E. G., Onipchenko, V. G., Peñuelas, J., Reich, P. B., Rillig, M. C., Sack, L., Shipley, B., Tedersoo, L., Valladares, F., van Bodegom, P., Weigelt, P., Wright, J. P., & Weigelt, A. (2020). Global Root Traits (GRooT)

Database. *Global Ecology and Biogeography 30*(1), 25-37. https://doi.org/10.1111/geb.13179

Iannucci, A., & Amato, M. (2021). Root morphology and shoot growth in seedlings of chia (*Salvia hispanica* L.). *Genetic Resources and Crop Evolution 68*(8), 3205-3217. https://doi.org/10.1007/s10722-021-01181-w

Intergovernmental Panel on Climate Change. (2023). *Climate change 2023: Synthesis report. Contribution of working groups I, II and III to the sixth assessment report of the Intergovernmental Panel on Climate Change* [core writing team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland. https://doi.org/10.59327/IPCC/AR6-9789291691647

Iversen, C. M., McCormack, M.L., Baer, J. K., Powell, A. S., Chen, W., Collins, C., Fan, Y., Fanin, N., Freschet, G. T., Guo, D., Hogan, J. A., Kou, L., Laughlin, D. C., Lavely, E., Liese, R., Lin, D., Meier, I. C., Montagnoli, A., Roumet, C., See, C. R., Soper, F., Terzaghi, M., Valverde-Barrantes, O. J., Wang, C., Wright, S. J., Wurzburger, N., Zadworny, M. (2021). *Fine-root ecology database (FRED): A global collection of root trait data with coincident site, vegetation, edaphic, and climatic data (Version 3).* Oak Ridge National Laboratory, U.S. Department of Energy. https://doi.org/10.25581/ornlsfa.014/1459186

Iversen, C. M., McCormack, M. L., Powell, A. S., Blackwood, C. B., Freschet, G. T., Kattge, J., Roumet, C., Stover, D. B., Soudzilovskaia, N. A., Valverde-Barrantes, O. J., Bodegom, P. M., & Violle, C. (2017). A global fine-root ecology database to address belowground challenges in plant ecology. *New Phytologist 215*, 15–26. https://doi.org/10.1111/nph.14486

Jiao, X., Yu, X.: Yuan, Y., & Li, J. (2022). Effects of vapor pressure deficit combined with different N levels on tomato seedling anatomy, photosynthetic performance, and N uptake. *Plant Science 324*, Article 111448. https://doi.org/10.1016/j.plantsci.2022.111448

Karlova, R., Boer, D., Hayes, S., & Testerink, C. (2021). Root plasticity under abiotic stress. *Plant Physiology 187*(3), 1057-1070. https://doi.org/10.1093/plphys/kiab392

Klimešová, J., & Herben, T. (2023). The hidden half of the fine root differentiation in herbs: Nonacquisitive belowground organs determine fine-root traits. *OIKOS 2023*(1), Article e08794. https://doi.org/10.1111/oik.08794

Klimešová, J., Martínková, J., & Ottaviani, G. (2018). Belowground plant functional ecology: Towards an integrated perspective. *Functional Ecology 32*(9), 2115-2126. https://doi.org/10.1111/1365-2435.13145

Khoshsokhan, F., Babalar, M., Salami, S. A., Sheikhakbari-Mehr, R., & Mirjalili, M. H. (2022). Rosmarinic acid production in hairy root cultures of *Salvia nemorosa* L. (Lamiaceae). *Biocatalysis and Agricultural Biotechnology 45*, Article 102494. https://doi.org/10.1016/j.bcab.2022.102494

Koehler, T., Wankmüller, F., Sadok, W., & Carminati, A. (2023). Transpiration response to soil drying versus increasing vapor pressure deficit in crops: Physical and physiological mechanisms and key plant traits. *Journal of Experimental Botany 74*(16), 4789-4807. https://doi.org/10.1093/jxb/erad221

Kong, D., & Fridley, J. D. (2019). Does plant biomass partitioning reflect energetic investments in carbon and nutrient foraging? *Functional Ecology 33*(9), 1627-1637. https://doi.org/10.111/1365-2435.13392

Kong, D., Ma, C., Zhang, Q., Li, L., Chen, X., Zeng, H., & Guo, D. (2014). Leading dimensions in absorptive root trait variation across 96 subtropical forest species. *New Phytologist 203*(3), 863-872. https://doi.org/10.1111/nph.12842

Kutschera, L., Lichtenegger, E., & Sobotik, M. (1992). *Wurzelatlas mitteleuropäischer Grünlandpflanzen, Band 2: Pteridophyta und Dicotyledoneae (Magnoliopsida) Teil 1: Morphologie, Anatomie, Ökologie, Verbreitung, Soziologie, Wirtschaft* [Root atlas

of Central European grassland plants, volume 2: Pteridophyta and Dicotyledoneae (Magnoliopsida) part 1: Morphology, anatomy, ecology, distribution, sociology, economy]. Gustav Fischer.

Langguth, J. R., Zadworny, M., Andraczek, K., Lo, M., Tran, N., Patrick, K., Mucha, J., Mueller, K. E., & McCormack, M. L. (2024). Gymnosperms demonstrate patterns of fine-root trait coordination consistent with the global root economics space. *The Journal of Ecology 112*(6), 1425-1439. https://doi.org/10.1111/1365-2745.14315

Larson, J. E., & Funk, J. L. (2016). Seedling responses to soil moisture and the identification of a belowground trait spectrum across three growth forms. *New Phytologist 210*(3), 827-838. https://doi.org/10.1111/nph.13829

Li, H., Liu, B., McCormack, M. L., Ma, Z., & Guo, D. (2017). Diverse belowground resource strategies underlie plant species coexistence and spatial distribution in three grasslands along a precipitation gradient. *New Phytologist 216*(4), 1140-1150. https://doi.org/10.1111/nph.14710

López, J., Way, D. A., & Sadok, W. (2021). Systemic effects of rising atmospheric vapor pressure deficit on plant physiology and productivity. *Global Change Biology 27*(9), 1704-1720. https://doi.org/10.1111/gcb.15548

Luo, H., Xu, H., Chu, C., He, F., & Fang, S. (2020). High temperature can change root system architecture and intensify root interactions of plant seedlings. *Frontiers in Plant Science 11*, Article 160. https://doi.org/10.3389/fpls.2020.00160

Lyu, D., Backer, R., & Smith, D. L. (2022). Three plant growth-promoting rhizobacteria alter morphological development, physiology, and flower yield of *Cannabis sativa* L. *Industrial Crops and Products 178*, Article 114583. https://doi.org/10.1016/j.indcrop.2022.114583

McCormack, M. L., Dickie, I. A., Eissenstat, D. M., Fahey, T. J., Fernandez, C. W., Guo, D., Helmisaari, H.-S., Hobbie, E. A., Iversen, C. M., Jackson, R. B., Leppälammi-Kujansuu, J., Norby, R. J., Phillips, R. P., Pregitzer, K. S., Pritchard, S. G., Rewald, B., & Zadworny, M. (2015). Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytologist 207*(3), 505-518. https://doi.org/10.1111/nph.13363

McCormack, M. L., Guo, D., Iversen, C. M., Chen, W., Eissenstat, D. M., Fernandez, C. W., Li, L., Ma, C., Ma, Z., Poorter, H., Reich, P. B., Zadworny, M., & Zanne, A. (2017). Building a better foundation: Improving root-trait measurements to understand and model plant and ecosystem processes. *New Phytologist 215*(1), 27-37. https://doi.org/10.1111/nph.14459

McCormack, M. L., Kaproth, M. A., Cavender-Bares, J., Carlson, E., Hipp, A. L., Han, Y., & Kennedy, P. G. (2020). Climate and phylogenetic history structure morphological and architectural trait variation among fine-root orders. *New Phytologist 228*(6) 1824-1834. https://doi.org/10.1111/nph.16804

Menzel, A., Yuan, Y., Matiu, M., Sparks, T., Scheifinger, H., Gehrig, R., & Estrella, N. (2020). Climate change fingerprints in recent European plant phenology. *Global Change Biology 26*(4), 2599-2612. https://doi.org/10.1111/gcb.15000

Mucha, J., Zadworny, M., Helmisaari, H.-S., Nihlgård, B., Repo, T., Żytkowiak, M., Małek, S., Reich, P. B., & Oleksyn, J. (2020). Fine root classification matters: Nutrient levels in different functional categories, orders and diameters of roots in boreal *Pinus sylvestris* across a latitudinal gradient. *Plant Soil 447*(1-2), 507-520. https://doi.org/10.1007/s11104-019-04395-1

Narazi-Montazer, J. (2023). Phenological patterns of five herbaceous plant species: A comprehensive analysis. [Unpublished bachelor thesis]. Department of Molecular Systems Biology, University of Vienna.

Nikolova, P. S., Bauerle, T. L., Häberle, K.-H., Blaschke, H., Brunner, I., & Matyssek, R. (2020). Fine-root traits reveal contrasting ecological strategies in European beech

and Norway spruce during extreme drought. *Frontiers in Plant Science 11*, Article 1211. https://doi.org//10.3389/fpls.2020.01211

Norby, R. J., & Iversen, C. M. (2017). Introduction to a virtual issue on root traits. *New Phytologist 215*(1), 5-8. https://doi.org/10.1111/nph.14522

Nordt, B., Hensen, I., Bucher, S. F., Freiberg, M., Primack, R. B., Stevens, A.-D., Bonn, A., Wirth, C., Jakuba, D., Plos, C., Sporbert, M., & Römermann, C. (2021). The PhenObs initiative: A standardised protocol for monitoring phenological responses to climate change using herbaceous plant species in botanical gardens. *Functional Ecology 35*(4), 821-834. https://doi.org/10.1111/1365-2435.13747

Palacio, S., & Montserrat-Martí, G. (2007). Above and belowground phenology of four Mediterranean sub-shrubs: Preliminary results on root–shoot competition. *Journal of Arid Environments 68*(4), 522-533. https://doi.org/10.1016/j.jaridenv.2006.07.008

Piao, S., Liu, Q., Chen, A., Janssens, I. A., Fu, Y., Dai, J., Liu, L., Lian, X., Shen, M., & Zhu, X. (2019). Plant phenology and global climate change: Current progresses and challenges. *Global Change Biology 25*(6), 1922-1940. https://doi.org/10.1111/gcb.14619

Primka, E. J., Adams, T. S., Buck, A., & Eissenstat, D. M. (2021). Topographical shifts in fine root lifespan in a mixed, mesic temperate forest. *PLOS ONE 16*(7), Article 0254672. https://doi.org/10.1371/journal.pone.0254672

Radville, L., McCormack, M. L., Post, E., & Eissenstat, D. M. (2016). Root phenology in a changing climate. *Journal of Experimental Botany 67*(12), 3617-3628. https://doi.org/10.1093/jxb/erw062

Seethepalli, A., Dhakal, K., Griffiths, M., Guo, H., Freschet, G. T., & York, L. M. (2021). RhizoVision Explorer: Open-source software for root image analysis and measurement standardisation. *RhizoVision Explorer.* https://www.rhizovision.com/manual

Seethepalli, A., & York, L. M. (2021). RhizoVision Explorer: Interactive software for generalized root image analysis designed for everyone (Version 2.0.3). *Zenodo*. https://doi.org/10.5281/zenodo.3747697

Slette, I. J., Hoover, D. L., Smith, M. D., & Knapp, A. K. (2023). Repeated extreme droughts decrease root production, but not the potential for post-drought recovery of root production, in a mesic grassland. *OIKOS 2023*(1), Article e08899. https://doi.org/10.1111/oik.08899

Sporbert, M., Jakubka, D., Bucher, S. F., Hensen, I, Freiberg, M., Heubach, K., König, A., Nordt, B., Plos, C., Blinova, I., Bonn, A., Knickmann, B., Koubek, T., Linstädter, A., Mašková, T., Primack, R. B., Rosche, C., Shah, M. A., Stevens, A.-D., Tielbörger, K., Träger, S., Wirth, C., & Römermann, C. (2022). Functional traits influence patterns in vegetative and reproductive plant phenology: A multi-botanical garden study. *New Phytologist 235*(6), 2199-2210. https://doi.org/10.1111/nph.18345

Steinaker, D. F., & Wilson, S. D. (2008). Phenology of fine roots and leaves in forest and grassland. *Journal of Ecology 96*(6), 1222-1229. https://doi.org/10.1111/j.1365-2745.2008.01439.x

Sun, S.-S., Liu, X.-P., Zhao, X.-Y., Medina-Roldánd, E., He, Y.-H., Lv, P., & Hu, H.-J. (2022). Annual herbaceous plants exhibit altered morphological traits in response to altered precipitation and drought patterns in semiarid sandy grassland, Northern China. *Frontiers in Plant Science 13*, Article 756950. https://doi.org/10.3389/fpls.2022.756950

Tocai Moţoc, A. C., Kokeric, T., Tripon, S., Barbu-Tudoran, L., Barjaktarevic, A., Cupara, S., & Vicas, S. I. (2023). *Sanguisorba minor* Scop.: An overview of its phytochemistry and biological effects. *Plants 12*(11), Article 2128. https://doi.org/10.3390/plants12112128

Torres, C. D., Magnin, A., Sabatier, S., Puntieri, J. G., & Caraglio, Y. (2022). Assessing coordinated intra-specific variation in root/shoot traits in two herbaceous species based on architecture and ontogeny. *Folia Geobotanica 57*, 167-180. https://doi.org/10.1007/s12224-022-09419-w

Torrion, J. A., Setiyono, T. D., Cassman, K. G., Ferguson, R. B., Irmak, S., & Specht, J. E. (2012). Soybean root development relative to vegetative and reproductive phenology. *Agronomy Journal 104*(6), 1702-1709. https://doi.org/10.2134/agronj2012.0199

University of Vienna. (2024, August 22). *PhenObs*. Botanical Garden of the University of Vienna. https://botanischergarten.univie.ac.at/en/science/research/phenobs/

Vescio, R., Abenavoli, R. M., Sorgonà, A. (2020). Single and combined abiotic stress in maize root morphology. *Plants 10*(1), Article 5. https://doi.org/10.3390/plants10010005

Wang, J., Defrenne, C., McCormack, M. L., Yang, L., Tian, D., Luo, Y., Hou, E., Yan, T., Li, Z., Bu, W., Chen, Y., & Niu, S. (2021). Fine-root functional trait responses to experimental warming: A global meta-analysis. *New Phytologist 230*(5), 1856-1867. https://doi.org/10.1111/nph.17279

Wang, P., Huang, K., & Hu, S. (2020). Distinct fine-root responses to precipitation changes in herbaceous and woody plants: A meta-analysis. *New Phytologist 225*(4), 1491-1499. https://doi.org/10.1111/nph.16266

Wang, B., McCormack, M. L., Ricciuto, D. M., Yang, X., & Iversen, C. M. (2023). Embracing fine-root system complexity in terrestrial ecosystem modelling. *Global Change Biology 29*(11), 2871-2885. https://doi.org/10.1111/gcb.16659

Wang, S., Wang, W., Wang, S., Yang, L., & Gu, J. (2021). Intraspecific variations of anatomical, morphological and chemical traits in leaves and absorptive roots along climate and soil gradients: A case study with *Ginkgo biloba* and *Eucommia ulmoides*. *Plant and Soil 469*(1-2), 73-88. https://doi.org/10.1007/s11104-021-05149-8

Weemstra, M., Kuyper, T. W., Sterck, F. J., & Umaña, M. N. (2023). Incorporating belowground traits: Avenues towards a whole-tree perspective on performance. *OIKOS 2023*(1), Article e08827. https://doi.org/10.1111/oik.08827

Weemstra, M., Valverde-Barrantes, O. J., McCormack, M. L., & Kong, D. (2023). Special issue: Root traits and functioning: From individual plants to ecosystems. *OIKOS 2023*(1), Article e09924. https://doi.org/10.1111/oik.09924

Weiwar, J. (2023). Phänologische Beobachtung und Untersuchung von vier Zeigerpflanzen [Phenological observation and investigation of four indicator plants]. [Unpublished bachelor thesis]. Department of Molecular Systems Biology, University of Vienna.

Xing, Y., Chen, M., Dao, J., Lin, L., Chen, C., Chen, Y., & Wang, Z. (2024). Fine-root morphology of woody and herbaceous plants responds differently to altered precipitation: A meta-analysis. *Forest Ecology and Management 552*, Article 121570. https://doi.org/10.1016/j.foreco.2023.121570

Ye, Z., Mu, Y., van Duzen, S., & Ryser, P. (2024). Root and shoot phenology, architecture, and organ properties: An integrated trait network among 44 herbaceous wetland species. *New Phytologist*, https://doi.org/10.1111/nph.19747

York, L. M. (2020). Plans for root scanning trays to use on flatbed scanners. *Zenodo*. https://doi.org/10.5281/zenodo.4122423

York, L. M. (2023). Setting up the Epson Expression 12000XL for root scanning. *Zenodo*. https://doi.org/10.5281/zenodo.7825851

Zhou, M., Gui, Y., Sheng, J., Yuan, Y., Zhang, W.-H., & Bai, W. (2022). Using anatomical traits to understand root functions across root orders of herbaceous species in a temperate steppe. *New Phytologist 234*(2), 422-434. https://doi.org/10.1111/nph.17978

Table S1 Measures of variability and normality for weather variables across all locations and years, with ANOVA or Kruskal-Wallis results by location.

								ANOVA			Kruskal-Wallis	
Variable	**Minimum**	**Maximum**	**Range**	**Std Dev**	**CV (%)**	**Shapiro-Wilk**	**p**	**F**	**p**	**η^2**	**H**	**p**
T_{avg}	9.961	14.269	4.308	1.298	11.2	0.882	0.041*				9.816	0.020*
T_{max}	14.222	18.044	3.822	1.086	6.7	0.973	0.879	5.112	0.017*	0.561		
Days <0°C	0	15	15	5.419	93.2	0.881	0.040*				4.137	0.247
Days 0-4.9°C	19	51	32	9.366	27.2	0.968	0.803	0.275	0.842	0.064		
Days 5-9.9°C	54	79	25	7.473	11.7	0.936	0.301	0.835	0.500	0.173		
Days 10-14.9°C	53	81	28	8.467	12.9	0.956	0.588	1.426	0.284	0.263		
Days 15-19.9°C	50	79	29	7.736	12.4	0.963	0.722	0.278	0.841	0.065		
Days 20-24.9°C	47	72	25	6.082	9.8	0.927	0.219	1.276	0.327	0.242		
Days 25-29.9°C	34	61	27	7.810	15.5	0.934	0.282	4.366	0.027*	0.522		
Days 30-34.9°C	6	35	29	9.054	52.1	0.893	0.063				10.906	0.012*
Days > 35°C	0	7	7	2.442	73.7	0.909	0.114				2.956	0.398
PRCP	354.700	777.400	422.700	132.210	23.6	0.953	0.545	0.974	0.437	0.196		
DWP total/year	172	228	56	18.543	9.0	0.889	0.053				4.895	0.180
DWP	10	22	12	3.357	22.8	0.949	0.477				2.547	0.467
RH	60.504	77.636	17.132	5.918	8.4	0.855	0.016*				10.743	0.013*
VP	9.606	10.700	1.094	0.333	3.3	0.887	0.050*				2.449	0.485

*p < 0.05, **p < 0.01, ***p < 0.001. Kruskal-Wallis tests were performed for all variables whose data was non-normally distributed. This includes variables classified as non-normal after a visual inspection, despite their Shapiro-Wilk result not reaching significance (see Section 3.2.2). The low reliability of Shapiro-Wilk in this setting is owed to the small sample size. The low number of significant values for ANOVA and Kruskal-Wallis indicate the weather to be quite similar across the sites regarding most of the parameters measured.

Table S2 Valid data, ranges, and measures of dispersion for phenology variables of *Salvia nemorosa* and *Sanguisorba minor* across all locations and years, with Kruskal-Wallis results by location.

								Shapiro-Wilk		Kruskal-Wallis	
Variable	**Species**	**Valid data (%)**	**Mean**	**Minimum**	**Maximum**	**Std Dev**	**CV (%)**	**Shapiro-Wilk**	**p**	**H**	**p**
IG	*S. nemorosa*	77.33	48.586	5	89	19.896	41.0	0.928	0.002**	18.236	0.001**
	S. minor	18.67	33.714	1	55	21.773	64.6	0.827	0.011*	5.925	0.052
FL	*S. nemorosa*	88.00	72.061	27	97	15.913	22.1	0.945	0.006**	13.854	0.008**
	S. minor	77.33	43.310	-8	90	25.444	58.7	0.955	0.032*	6.653	0.155
FF	*S. nemorosa*	98.67	155.162	131	203	12.746	8.2	0.902	<0.001***	3.614	0.461
	S. minor	98.67	119.297	105	137	8.415	7.1	0.960	0.019*	2.013	0.733
PF	*S. nemorosa*	78.67	162.678	152	190	8.587	5.3	0.808	<0.001***	19.785	<0.001***
	S. minor	74.67	130.857	114	153	9.196	**7.0**	0.968	0.142	n/a	n/a
EPF	*S. nemorosa*	78.67	179.407	159	218	10.378	5.8	0.864	<0.001***	8.643	0.071
	S. minor	80.00	144.733	121	163	10.338	**7.1**	0.936	0.004**	9.535	0.049*
EF	*S. nemorosa*	94.67	264.789	180	372	65.873	24.9	0.886	<0.001***	12.348	0.015*
	S. minor	97.33	183.986	145	266	32.666	**17.8**	0.786	<0.001***	31.594	<0.001***
FD	*S. nemorosa*	94.67	110.070	5	217	66.485	60.4	0.890	<0.001***	7.371	0.118
	S. minor	97.33	64.767	20	147	34.210	52.8	0.856	<0.001***	22.844	<0.001***
FFr	*S. nemorosa*	90.67	185.779	166	246	15.800	8.5	0.797	<0.001***	21.304	<0.001***
	S. minor	98.67	161.595	141	182	9.193	5.7	0.937	0.001**	20.865	<0.001***
SS	*S. nemorosa*	90.67	184.794	145	310	28.631	15.5	0.809	<0.001***	13.126	0.011*
	S. minor	66.67	178.480	145	254	27.978	15.7	0.877	<0.001***	6.574	0.160
S50	*S. nemorosa*	92.00	280.304	208	356	41.435	14.8	0.964	0.043*	36.840	<0.001***
	S. minor	29.33	279.727	154	358	62.132	22.2	0.934	0.147	n/a	n/a
VP	*S. nemorosa*	78.67	113.949	56	239	35.868	31.5	0.915	<0.001***	21.735	<0.001***
	S. minor	42.67	123.875	76	163	24.290	19.6	0.961	0.297	n/a	n/a

*p < 0.05, **p < 0.01, ***p < 0.001. To assess location-specific differences in phenology, Kruskal-Wallis tests were conducted for the non-normally distributed phenological variables. The results are displayed in this table. ANOVA results for the normally distributed variables were also conducted but are disregarded in the table due to insignificance.

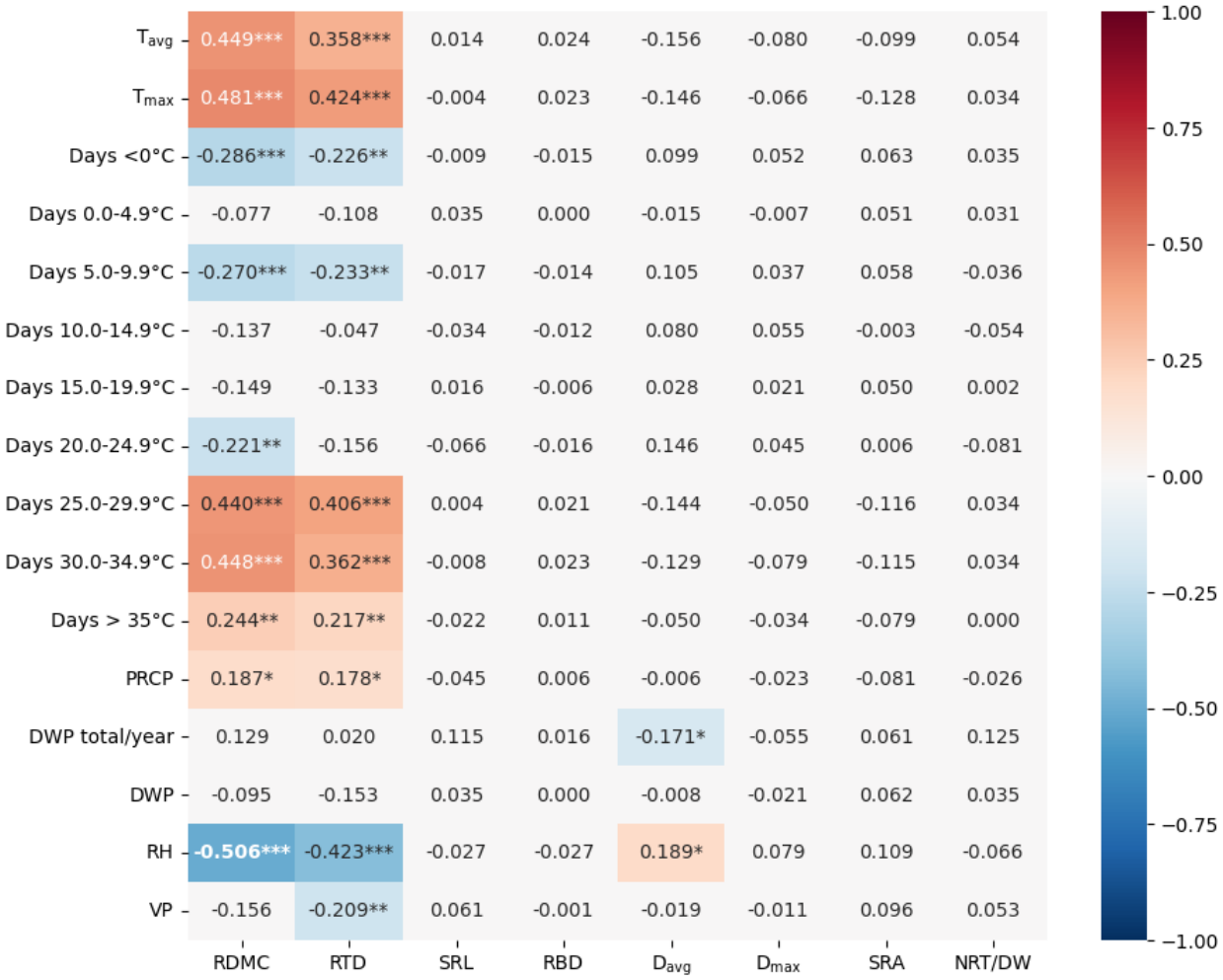

Figure S1 Spearman's ρ values for root variables with weather parameters of all four years, across both species (n = 39). Because RDMC and RTD do not differ significantly between *Salvia nemorosa* and *Sanguisorba minor* (see Table 4), their joint correlations are particularly interesting. Values reaching significance (*p < 0.05, **p < 0.01, ***p < 0.001) are coloured, with values above 0.5 or below -0.5 in bold. Negative correlations are displayed in blue, positive correlations in red. Non-significant values are included but unshaded.

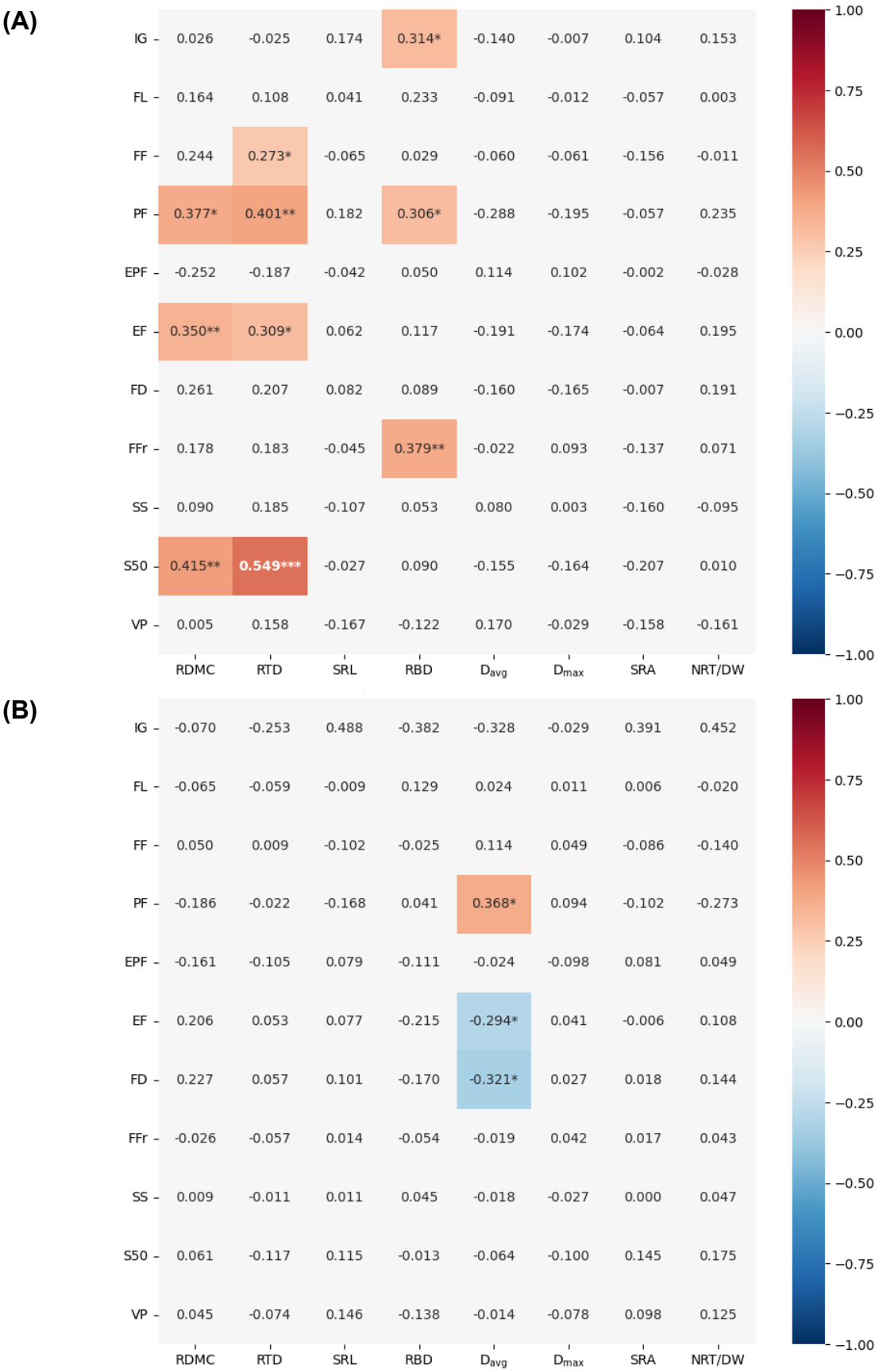

Figure S2 Spearman's ρ values for root variables with phenological variables of all four years. **(A)** *Salvia nemorosa* (n = 20) and **(B)** *Sanguisorba minor* (n = 19). Values reaching significance (*p < 0.05, **p < 0.01, ***p < 0.001) are coloured, with values above 0.5 or below -0.5 in bold. Negative correlations are displayed in blue, positive correlations in red. Non-significant values are included but unshaded.

Two herbaceous species, their root systems, phenology, and climatic associations across botanical gardens

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Recent confirmation of anthropogenic influence on temporal shifts in plants' developmental patterns supports the suitability of phenology as a bio-indicator for climate change (Menzel et al. 2020). Within this context, the project "PhenObs", an international network of botanical gardens, is investigating herbaceous plants' phenology. Due to such endeavours, data exist on aboveground plant development, there is however a lack of information on root growth and its connection to phenology (Piao et al. 2019). For the present analysis, 105 individuals each of *Salvia nemorosa* and *Sanguisorba minor,* grown and monitored from 2020 to 2023 in botanical gardens in Vienna, Jena, Halle, Berlin, and Chicago, are used to investigate the relationships between climatic conditions, root system characteristics, and aboveground phenology. Aboveground data collected over the course of several years by "PhenObs" participants are complimented with root analyses done at the end of 2023, as well as climate data recorded by meteorological stations close to the respective botanical gardens. Preliminary results suggest that phenological data can hardly be correlated to climate data on a coarse yearly scale, with only very weak correlations between vegetation period and precipitation ($r=0.361$, $p<0.001$), and flowering duration and precipitation ($r=0.241$, $p<0.004$). Results also reveal strong variations in Viennese plants' root systems. High consistency across the sample is found only in root dry matter content in *Salvia nemorosa* and mean root diameter in *Sanguisorba minor*, while root branching frequency shows little variation for both species. Further analyses will reveal whether similar root traits occur in other gardens, whether an influence of climate on root development can be found, and whether above- and belowground development of plants correlate to each other. Combining these results, we can estimate the suitablility of *Salvia nemorosa* and *Sanguisorba minor* as bioindicators for climatic conditions.

German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig. 2023. "Über PhenObs". *PhenObs.* [Online] https://www.idiv.de/de/web/phenobs.html (30.10.2023)

Menzel, A.; Yuan, Y.; Matiu, M.; Sparks, T.; Scheifinger, H.; Gehrig, R.; Estrella, N. 2020. "Climate change fingerprints in recent European plant phenology". *Global Change Biology* 26, 2599-2612. https://doi.org/10.1111/gcb.15000

Piao, S.; Liu, Q.; Chen, A.; Janssens, I. A.; Fu, Y.; Dai, J.; Liu, L.; Lian, X.; Shen, M.; Zhu, X. 2019. "Plant phenology and global climate change: Current progresses and challenges". *Global Change Biology* 25, 1922-1940. https://doi.org/10.1111/gcb.14619

Two herbaceous plants and their root systems' association to phenology and weather across botanical gardens

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Introduction

Salvia nemorosa, n = 20

Sanguisorba minor, n = 19

Root growth is severely understudied due to challenges in root investigation (Freschet et al. 2021), as are the influence of abiotic factors such as weather on root formation, and the (a)synchrony of plant development above and below the ground.

As part of the PhenObs project (iDiv 2024), four European botanical gardens harboured plants from the same original populations under similar conditions. Standardised monitoring of phenology (Nordt et al. 2021) for four years was followed by a root harvest at the end of 2023.

Objectives & Methods

Research questions

1. What are defining characteristics of the root systems of *Salvia nemorosa* and *Sanguisorba minor* in the botanical gardens in Berlin, Halle, Jena, and Vienna?
2. How do root traits relate to the weather and aboveground phenology across the different sites and years?

Methods

- Root harvest, scanning, and analysis using RhizoVision Explorer (Seethepalli & York 2021)
- Phenology data retrieval from PhenObs
- Weather data retrieval from DWD Climate Data Center (2024) and GeoSphere Austria data hub (2024)
- Exploratory data analysis for differences and correlations between traits, species, and gardens

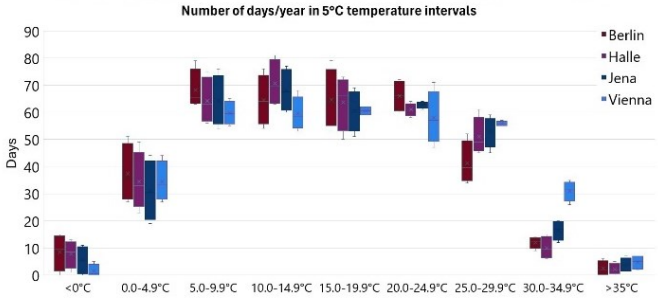
Table 1 Overview of variables used for calculations

		Variable name	Abbr.	Unit
Roots	N = 39	Root dry matter content	RDMC	mg/mg
		Root tissue density	RTD	mg/mm³
		Specific root length	SRL	mm/mg
		Root branching density	RBD	mm⁻¹
		Diameter (average, maximum)	Davg, Dmax	mm
		Specific root area	SRA	mm²/mg
		Number of root tips per dry weight	NRT/DW	mg⁻¹
Weather	2020-2023	Average daily temperature	Tavg	°C
		Averages of maximum daily temperatures	Tmax	°C
		Numbers of days with maximum daily temperature within a 5°C interval		days/year
		Precipitation	PRCP	mm
		Number of days without precipitation	DWP total/year	days/year
		Longest streak of days without precipitation	DWP	
		Average relative humidity	RH	%
		Average vapor pressure	VP	hPa
Phenology	N = 210	Initial growth	IG	day of year
		First leaf	FL	day of year
		First flower	FF	day of year
		Peak flowering	PF	day of year
		End of peak flowering	EPF	day of year
		End of flowering	EF	day of year
		Flowering duration	FD	days
		First fruit	FFr	day of year
		Start of senescence	SS	day of year
		Vegetation period	VP	days
		50% senescence	S50	day of year

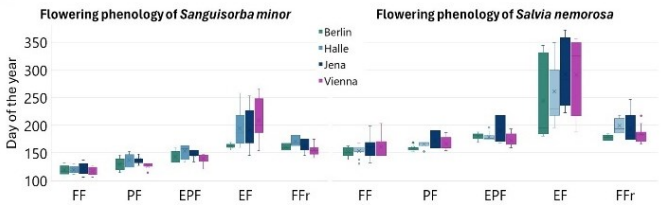
Segment-based root classification and selection of order 1, 2, and 3 fine roots

Results

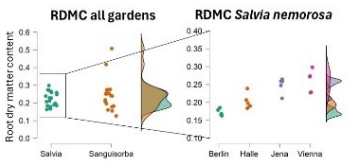
Weather

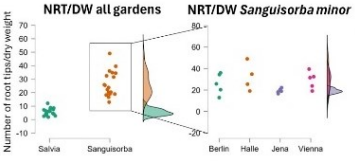


Plant phenology



Roots

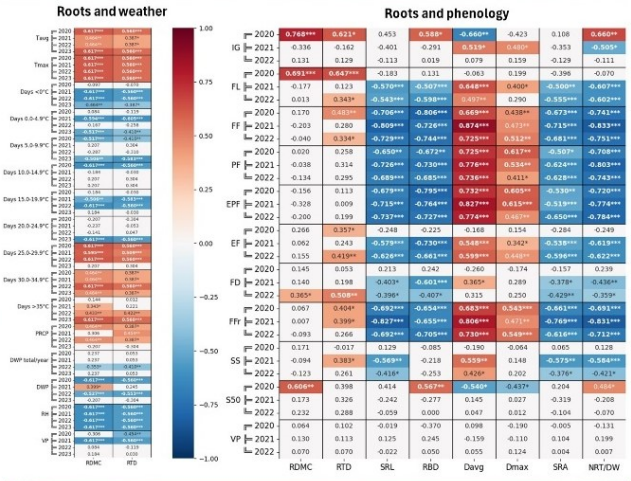




Root mass correlates with weather. Root length, branching density, diameter, area, and root tips correlate with plant phenology.

Most weather variables differ ($p<0.006$) between the gardens.
Phenology data are most complete for FF to SS, and the majority of traits (except FL, FF, and EPF) differ ($p<0.037$) between the gardens.
RDMC and RTD for *Salvia nemorosa*, and Davg for *Sanguisorba minor* differ between locations ($p<0.022$). Other traits differ between species (SRL, RBD, Davg, Dmax, SRA, NRT/DW, $p<0.001$) but not between gardens.
Spearman's rank for all phenology and weather variables with all root variables suggests a correlation pattern of RDMC and RTD with weather variables, while SRL, RBD, Davg and Dmax, SRA, and NRT/DW seem more tightly linked to (reproductive) phenology.

Correlations



Conclusion

For both species, the most characteristic root trait showing the least dispersion and variation between gardens is the root branching density.
Significant differences in weather conditions and aboveground plant development across the gardens allow a calculation of links between weather, phenology, and root morphology and growth. For the association of phenology and roots, the direction of influence remains a promising subject of future research.
The phenological development and root systems of *Salvia nemorosa* and *Sanguisorba minor* differ considerably. Nevertheless, their root traits' striking correlations with phenology and weather underline the importance of understanding all three factors for an overall comprehension of herbaceous plants' development.

References

DWD Climate Data Center. 2024. *CDC - Climate Data Center.* https://cdc.dwd.de/portal/202209231028/

Freschet, G. T., et al. 2021. A starting guide to root ecology: Strengthening ecological concepts and standardising root classification, sampling, processing and trait measurements. *New Phytol.* 232(3): 973-1122.

GeoSphere Austria data hub. 2024. *Stationsdaten.* https://data.hub.geosphere.at/

German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig. 2024. *PhenObs.* https://www.idiv.de/de/web/phenobs.html

Nordt, B., et al. 2021. The PhenObs initiative: A standardised protocol for monitoring phenological responses to climate change using herbaceous plant species in botanical gardens. *Funct. Ecol.* 35, 821-834. https://doi.org/10.1111/1365-2435.13747

Seethepalli, A.; York, L. M. 2021. *RhizoVision Explorer – Interactive software for generalized root image analysis designed for everyone* (Version 2.0.3). https://doi.org/10.5281/zenodo.3747697

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PhenObs Botanical Gardens as a Global Phenological Observation Network

APPN Austrian Plant Phenotyping Network