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Phenotypic and Phytochemical Variability Among Four Populations of *Hedeoma multiflora* Benth. (Tomillito de las Sierras) Native to the Province of Córdoba—In Situ Evaluation

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Simple Summary

Hedeoma multiflora Benth. is an endangered aromatic and medicinal wild species used in traditional medicine. The cultivation of native species represents a small and untapped sector within the agricultural industry, offering a viable alternative for accessing new markets with differentiated products. The market is supplied by rural residents, who collect products from their habitat and sell them informally, uprooting them, which prevents their development. Excessive and inappropriate extraction of natural populations leads to their decline and loss of genetic diversity. Phenotypic and phytochemical variability was evaluated in four populations of *H. multiflora* in the province of Córdoba to develop conservation strategies and cultivation protocols. Forty individuals from each population were sampled to analyze morphological, chemical, phenological, edaphological, and climatic variables. Significant differences were observed between populations: Tulumba had taller plants and larger leaves, while Bialet Massé had the highest number of internodes. Essential oil yield ranged from 1.01% to 2.10%, with Ongamira having the highest content. Phytochemical analysis revealed two chemotypes: pulegone-dominant (Bialet Massé and Río Cuarto) and menthone-dominant (Ongamira and Tulumba). Phenological patterns differed significantly, with Ongamira showing the greatest reproductive synchronization. Soil organic matter content significantly influenced morphological and chemical traits. The high variability among populations allows for the preservation of genetic diversity for domestication and conservation strategies.

Abstract

Hedeoma multiflora Benth. is an endangered aromatic and medicinal wild species native to Argentina, widely used in traditional medicine, whose cultivation represents a small and untapped sector within the agricultural industry. Current market supply relies on wild harvesting practices by rural communities, leading to population decline and genetic diversity loss through inappropriate extraction methods, including uprooting during suboptimal phenological stages. This study evaluated phenotypic and phytochemical variability in four populations of *H. multiflora* in the province of Córdoba to develop conservation strategies and cultivation protocols. Forty individuals were sampled from each population to analyze morphological, chemical, phenological, edaphological, and climatic variables. Significant differences were observed between populations: Tulumba had the tallest plants and largest leaves, while Bialet Massé had the highest number of internodes. Essential oil yield ranged from 1.01% to 2.10%, with Ongamira having the highest content.

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Academic Editor: Joel Heinen

Received: 12 June 2025
Revised: 8 August 2025
Accepted: 18 August 2025
Published: 25 August 2025

Citation: Turco, F.R.; Chaves, A.G.; de Blas, F.J.; Torres, L.E. Phenotypic and Phytochemical Variability Among Four Populations of *Hedeoma multiflora* Benth. (Tomillito de las Sierras) Native to the Province of Córdoba—In Situ Evaluation. *Wild* **2025**, *2*, 33. https://doi.org/10.3390/wild2030033

Correction Statement: This article has been republished with a minor change. The change does not affect the scientific content of the article and further details are available within the backmatter of the website version of this article.

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Phytochemical analysis revealed two chemotypes: pulegone-dominant (Bialet Massé and Río Cuarto) and menthone-dominant (Ongamira and Tulumba). Phenological patterns differed significantly, with Ongamira showing the greatest reproductive synchronization. Soil organic matter content significantly influenced morphological and chemical traits. The high variability among populations underscores the importance of preserving genetic diversity for domestication and conservation strategies.

Keywords: conservation; cultivation strategies; essential oils; *Hedeoma multiflora*; phytochemical diversity

1. Introduction

In Argentina, particularly within the province of Córdoba, there exists a widespread commercial network for aromatic, medicinal, and culinary species, encompassing numerous native botanical taxa. In the province of Córdoba, by provision of Law No. 10,337, the Ministry of Public Services, through the Secretariat of Environment and Climate Change in the province of Córdoba as the Enforcement Authority, regulates the conservation of biological diversity, establishes the thresholds for the sustainable use of natural resources, and authorizes access to them, in accordance with articles 124 and 41 of the Argentine National Constitution, article 66 of the Constitution of the Province of Córdoba and Law No. 10,208 (Environmental Policy Law of the province of Córdoba); within the mentioned ex situ protected species are *Minthostachys vertillata* (Griseb) Epling., *Baccharis crispa* (Spreng.), *Salimaneae integrifolia* (Griseb.) and *Hedeoma multiflora* Benth., among others. However, the native plants are predominantly harvested from natural populations by rural collectors and subsequently distributed through informal commercial channels. This extractive activity, generally carried out without the corresponding authorization, frequently occurs without consideration of the plants' phenological stages, with specimens often being completely uprooted, a practice that significantly impedes natural regeneration processes [1]. Such unsustainable harvesting methodologies contribute to substantial germplasm depletion through genetic erosion, representing a significant conservation challenge for regional biodiversity. This phenomenon has been well documented in multiple scientific studies, highlighting the urgent need to implement sustainable harvesting protocols, preserve local practices, and implement conservation strategies for several species, such as shrubs, *Minthostachys mollis*, *Julocroton argenteus*, *Baccharis crispa*, *Trixis divaricate* subsp. *discolor*, *Aloysia gratissima*, *Lippia turbinate*, and *Baccharis articulata*, and for two herbs, *Hedeoma multiflora* and *Passiflora caerulea* [2–8].

H. multiflora, commonly known as "tomillito de las sierras," is an aromatic and medicinal species native to the mountains of Córdoba, which is in a precarious state of conservation, being among taxa that face significant ecological vulnerability due to anthropogenic pressures such as land-use change and unsustainable collection practices [4–10].

H. multiflora is characterized as a highly aromatic perennial herbaceous plant that develops in compact clusters reaching up to 25 cm tall, featuring numerous ascending stems. The species exhibits oblong–linear leaves, which are sessile, pubescent, and punctate. Its floral morphology includes axillary flowers with tubular calyces displaying five teeth and bilabiate corollas in lilac or blue coloration. The reproductive structures produce fruits comprising four brown achenes embedded within a persistent calyx [11,12]. The abaxial surfaces of its leaves, green stems, and flowers feature simple glandular trichomes that secrete essential oil, whose main chemical constituents include L-limonene, menthone, and pulegone [12–16].

This aromatic herb is harvested from its natural habitat due to its valuable stimulating and digestive properties [16]. Extensive ethnobotanical research has documented its multifaceted therapeutic applications, particularly its antioxidant, cytotoxic, and gastroprotective activities [17–20]. Additionally, *H. multiflora* has gained prominence in complementary medical paradigms, including phytotherapy and aromatherapy, demonstrating its versatility in traditional and contemporary healing practices [14,16,21–23]. Beyond its medicinal applications, the species holds considerable commercial value in the food industry, where it serves as a key ingredient in the preparation of herbal bitters and appetizers, further emphasizing its economic and cultural significance in the region [24,25]. The excessive and unregulated extraction of *H. multiflora* throughout its natural distribution range has rendered it a threatened species. This conservation status stems from multiple factors, including population decline, genetic diversity erosion, and habitat degradation, leading to its classification as endangered [1,4,16,26]. Furthermore, according to the Conservation Priority Index for medicinal plants established by Martínez et al. [4], *H. multiflora* occupies the foremost position among medicinal herbs requiring priority conservation efforts in Córdoba. This designation underscores the critical need for implementing effective conservation strategies to ensure the species' long-term viability in its native ecosystem.

Genetic resource conservation encompasses both preservation and sustainable utilization, safeguarding the inherent variability that is fundamental to species maintenance. Analyzing the phenotypic variability within a species facilitates the establishment of both in situ and ex situ conservation strategies [27,28] and constitutes a central component in genetic diversity investigations. This approach aligns with established conservation frameworks and contemporary scientific consensus regarding biodiversity preservation methodologies [29,30].

To establish a rational and sustainable utilization framework for *H. multiflora* and implement an effective production system, a comprehensive understanding of the existing variability within natural populations is a prerequisite to initiating the domestication process [31,32]. For all the above reasons, the objective of this investigation was to assess phenotypic and phytochemical variability among four populations of *H. multiflora* native to the Province of Córdoba, evaluated in their natural growth area. This systematic evaluation will provide essential baseline data for developing conservation strategies and cultivation protocols that maintain the species' genetic integrity while facilitating its sustainable commercial production.

2. Materials and Methods

2.1. Sampling Sites

During the autumn seasons of 2019 and 2021, an in situ evaluation of *H. multiflora* specimens was conducted across four native populations from different locations within the province of Córdoba, Argentina. The sampling sites, representative of the species' natural distribution range, were selected based on comprehensive bibliographic references [12], consultations with key informants, and herbarium records. In 2019, a scientific collection permit was obtained to access the Bialet Massé and Río Cuarto populations (GOBDIGI-248548111-921). Following the interruption of field activities due to the COVID-19 pandemic during 2020, research resumed in 2021 when an additional scientific collection permit was secured to access the Ongamira and Tulumba populations (GOBDIGI-248548111-921 annexed). These locations were sufficiently geographically separated to ensure reproductive, genetic isolation and heterogeneity in edaphoclimatic conditions [8,30,33]. Table 1 presents the geographical coordinates (longitude and latitude) and elevation (meters above sea level) for each population, as recorded using Global Positioning System technology and the location of the sample sites in the province of Córdoba (Figure 1).

Table 1. Geographic coordinates and herbarium accession information for the four native populations of Hedeoma multiflora identified and evaluated in this study.

Population	Department	Altitude *	Latitude	Longitude	Herbarium ID **
A—Bialet Massé	Punilla	688	31°18′26.7″ S	64°28′00.2″ W	2364 CS ACOR
B—Río Cuarto	Río Cuarto	791	32°56′53.5″ S	64°50′55.2″ W	2366 CS ACOR
C—Ongamira	Ischilín	1164	30°46′29.8″ S	64°23′52.3″ W	2367 CS ACOR
D—Tulumba	Tulumba	809	30°22′01.4″ S	64°08′56.6″ W	2365 CS ACOR

* meters above sea level; ** herbarium identification.



Figure 1. Geographic location of the sampling sites for wild populations of *Hedeoma multiflora* in the province of Córdoba. The map was generated using https://earth.google.com/ (accessed on 6 August 2025).

At least forty plants were randomly sampled from each population, with a minimum inter-specimen distance of one meter maintained to ensure the assessment of distinct individuals rather than clonal ramets [6,30,33]. Seed material was collected from each specimen, and four representative plants from each population were botanically identified and deposited in the Herbarium of the Facultad de Ciencias Agropecuarias de la Universidad Nacional de Córdoba (ACORD), as documented in Table 1. This methodological approach facilitated a robust analysis of interpopulation variability while adhering to standard protocols for botanical sampling and documentation.

To access the different sampling sites, the Authorization for Scientific Collection and Use of Biological Material (Ref. Note No.: GOBDIGI-248548111-921) was granted by the Environment Secretariat of the province of Córdoba, in accordance with the provisions of the protocol for access to biological resources of the province of Córdoba (2021).

2.2. Climatic and Edaphic Characterization

For the climatic characterization of each sampling location, data were obtained from the Omixom monitoring network's meteorological stations (https://newmagya.omixom.com). In addition, soil samples from each population's growth site were collected and analyzed at the Soil and Water Laboratory (LABSA) of the Facultad de Ciencias Agropecuarias de la Universidad Nacional de Córdoba to determine edaphic characteristics. Sampling

depth varied according to terrain conditions: 0–5 cm in highly stony areas and 0–20 cm in sandier substrates.

2.3. Plant Characterization

The morphological, phenological, and chemical characterization was conducted following methodologies established by Ojeda [3], Chaves [34], Massuh [31], Brunetti [6], and Brunetti et al. [8]. The following traits were recorded: plant height (measured as the length of the longest branch) (PH) (cm), number of branches per plant (BN) (n°. plant^{-1}), internode number (measured as the number of internodes in the longest branch) (IN) (n°. plant^{-1}), internode length (measured as the length of internodes in the longest branch) (IL) (cm), and diameter of base longest branch (DLB) (mm). Plants' growth habits, phenological stages, and flower color were also recorded [8,35,36].

To determine leaf length (Leaf-L) (cm), leaf width (Leaf-W) (cm), and leaf area (Leaf-A) (cm^2), fully expanded leaves from the middle third of the longest branch of each plant were collected and digitally scanned [37]. Image processing was performed using ImageJ (Version 1.54p) software [38] to quantify leaf parameters. The Leaf-L/Leaf-W ratio (Leaf L/W) was also estimated as the quotient between the leaf length and leaf width [37].

For essential oil content (EO) (%) assessment, three samples of dry leaves (5 g each) were extracted from each studied population. Oil extraction was performed via hydrodistillation, using a modified Clevenger apparatus, for 40 min. Essential oil content was subsequently expressed as ml per 100 g of dry material. The qualitative–quantitative analysis of the chemical composition of the essential oils was carried out using Perkin Elmer model Clarus 580-SQ8 equipment Serial N° 648N7021501 (PerkinElmer, Inc., Waltham, MA, USA). The data were obtained using the TurboMass 6.1.0 program, and the identification of the peaks was carried out by comparison with the spectra of the libraries of the NIST MS Search 2.0 program.

Finally, seeds from each sampled plant were collected, identified, and preserved at 4 °C under refrigeration [3,34] for subsequent investigations.

2.4. Statistical Analysis

All results obtained for each measured variable were analyzed using InfoStat software Version 2020 [39].

Differences among populations for each morphological variable evaluated were assessed using parametric Analysis of Variance (ANOVA). Mean values between populations were compared using the DGC posteriori test [40].

The chi-square test was also used to determine whether there is an association between the phenological state of the plants and their site of origin.

Principal Component Analysis (PCA) was employed to evaluate the discriminatory potential of morphological and edaphic traits across plant populations. This analysis identified variables with the greatest discriminant weight, explaining the observed phenotypic variability among the studied populations [8,30,35,41].

A Generalized Procrustes Analysis (GPA) was conducted to assess the degree of consensus among configurations derived for each population based on morphological and edaphic traits. This analytical approach aimed to determine whether these variables maintained consistent discriminant value across diverse growing environments [30,35,42].

3. Results

3.1. Climatic and Edaphic Characterization

Climatic data for the populations were obtained from various meteorological stations: Bialet Massé from the Cosquín Agricultura Cba station (30133), Ongamira from

the APRHI—Colonia Hogar (West) station (30084), Río Cuarto from the FAV-UNRC Agricultura Cba station (30262), and Tulumba from the Dean Funes Agricultural Cba station (30071) (https://newmagya.omixom.com). Average temperature and precipitation data recorded for each sampling site are presented in Table 2.

Table 2. Mean temperature and precipitation values recorded across sampling sites.

Population	Jan-Max (°C)	Jan-Min (°C)	Jul-Max (°C)	Jul-Min (°C)	AAT (°C)	AAP * (mm)
Bialet Massé	29.9	16.1	18.7	3.6	17.1	71.2
Río Cuarto	28.5	16.2	17.2	1.6	15.9	52.8
Ongamira	25.0	14.8	16.2	7.1	15.7	41.1
Tulumba	31.1	18.4	21.7	5.1	19.1	44.3

Jan-Max: January average maximum temperature; Jan-Min: January average minimum temperature; Jul-Max: July average maximum temperature; Jul-Min: July average minimum temperature; AAT: average annual temperature; AAP: average annual precipitation for each sampling site. * data taken from meteorological stations close to the sampling sites.

Table 3 presents the soil chemical parameters recorded at four *H. multiflora* sampling sites. It was observed that Bialet Massé stands out with the highest content of organic matter (OM) and organic carbon (OC), indicating that it is a soil with greater fertility and nutrient retention capacity. Río Cuarto and Tulumba presented intermediate values of OM and OC, with Tulumba being somewhat richer in organic matter, while Ongamira showed the lowest values of these parameters, indicating that these are soils with lower organic input.

Table 3. Chemical analysis of soil from evaluated *Hedeoma multiflora* populations.

Population	OM (%)	OC (%)	tN (%)	C:N ratio	pH	EC (dS/m)
Bialet Massé	7.3	4.2	0.3	14.0	7.6	0.5
Río Cuarto	2.5	1.4	0.1	11.2	7.3	0.9
Ongamira	2.0	1.1	0.1	10.4	7.8	0.4
Tulumba	3.4	1.9	0.1	12.5	7.4	0.4

OM: organic matter; OC: organic carbon; tN: total nitrogen; C:N ratio: carbon–nitrogen ratio; EC: electric conductivity.

The total nitrogen content (tN) follows the same trend as organic matter, where Bialet Massé presented substantially higher values, followed by Tulumba, Río Cuarto, and Ongamira. Regarding the carbon/nitrogen ratio (C:N), all sites presented adequate values, indicating good mineralization of organic matter. The recorded pH suggests that all four sampling sites featured calcareous and slightly alkaline soils, with Ongamira being the most alkaline, followed by Bialet Massé, Tulumba, and Río Cuarto. And the electrical conductivity (EC) showed a low value (<1.5 dS/m) at all four sampling sites.

When analyzing the relationships between soil variables, annual average temperature and precipitation, and four sampling locations through Principal Component Analysis (PCA), it was observed that 84% of the total variability can be explained by the first factorial plane (PC1 and PC2) (Figure 2). At the level of the first principal component (PC1), which explains 61.7% of the total variability, the variables with greater weight were the C:N ratio, organic matter, organic carbon and total nitrogen content, and annual average precipitation. The second principal component, which explains 22.3% of the total variability, and the variables with greater inertia on this axis were pH and electric conductivity. It is interesting to note that altitude explains part of the variability observed between populations and shows the same inertia at the level of both axes, PC1 and PC2.

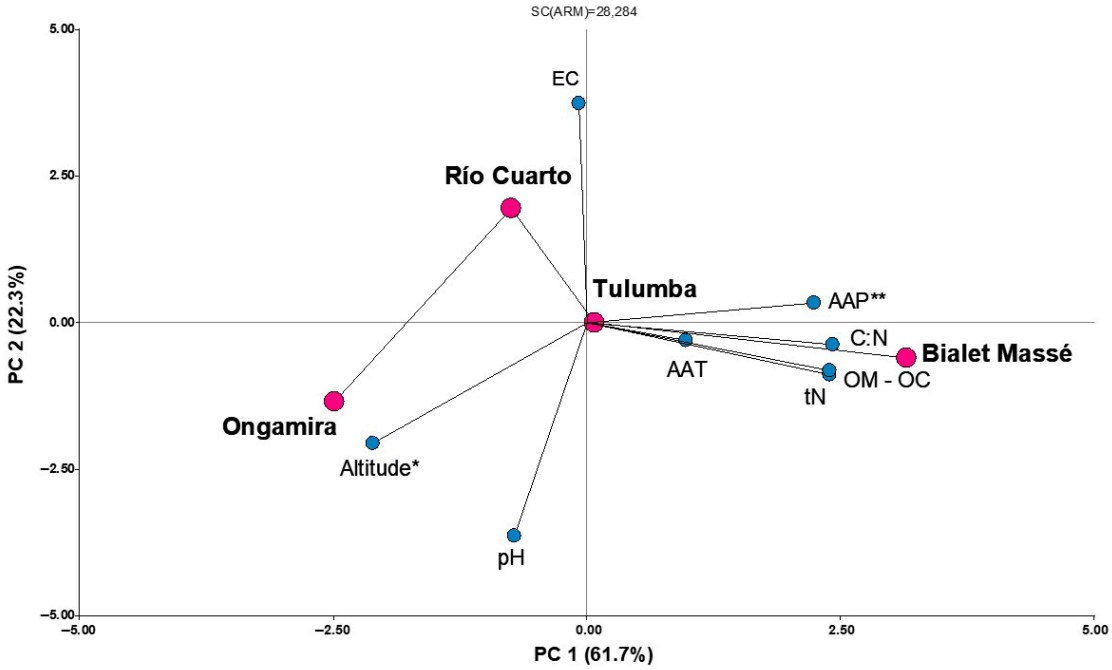

Figure 2. Biplot plane formed by the first two principal components (PC1 and PC2), where the dots represent *Hedeoma multiflora* sampling sites, and the vectors represent soil and climate variables. OM: organic matter content (%); OC: organic carbon content (%); tN: total nitrogen content (%); C:N: carbon–nitrogen ratio; EC: electric conductivity (dS/m); AAT: annual average temperature (°C); AAP: average annual precipitation for each sampling site (mm). * meters above sea level. ** data taken from meteorological stations close to the sampling sites.

The sampling locations showed clear separation: Bialet Massé was strongly associated with higher percentages of organic matter, organic carbon and total nitrogen, higher C:N, and higher precipitation values; Río Cuarto correlated positively with electrical conductivity; Tulumba showed intermediate characteristics among the soil parameters; and Ongamira showed a strong association with altitude and presented lower values for most of the edaphic and climatic variables measured.

3.2. Plant Characterization

Regarding plant growth habits and flower color, no differences were observed between the four populations, with all sampled specimens showing erect growth habits and lilac flowers (Figure 3).

Significant differences among the four evaluated populations were observed for the ten variables summarized in Table 4. The population of Río Cuarto showed the lowest plant height mean value, which differed significantly from the rest of the populations, followed by the populations of Bialet Massé and Ongamira (which do not differ significantly among them), and the population of Tulumba, which showed a significant highest mean value for this variable. Significant differences were also observed between the populations in terms of the number of branches per plant, where the populations of Tulumba and Ongamira exceeded the populations of Bialet Massé and Río Cuarto.

Considering the number and length of internodes, the population of Bialet Massé differed significantly from the rest of the populations by presenting the highest number of internodes (43) and the shortest ones (0.3 mm). The population of Río Cuarto also differed significantly from the rest of the populations by presenting the lowest number of internodes (26) and an intermediate internode length (0.4 mm), while the populations of Ongamira and Tulumba showed an average number of internodes of 32 and the highest internode

length (on average 0.5 mm) and did not differ significantly among themselves. Regarding the diameter of the longest branch, the Ongamira population showed the highest value of this variable, differing significantly from the Bialet Massé, Río Cuarto, and Tulumba populations, which did not differ among themselves.



Figure 3. All sampled specimens of *Hedeoma multiflora* showed erect growth habits (**a**) and lilac flowers (**b**).

Table 4. Mean values and coefficient of variation of 10 morphological characteristics of *Hedeoma multiflora* evaluated on native plants at four sampling sites.

	Population											
	Bialet Massé			**Río Cuarto**			**Ongamira**			**Tulumba**		
	Mean		**CV ***	**Mean**		**CV ***	**Mean**		**CV ***	**Mean**		**CV ***
PH (cm)	13.8 ± 0.5	b	25.1	10.4 ± 0.4	a	37.4	15.0 ± 0.7	b	30.4	18.5 ± 0.7	c	25.4
BN (n∘. plant^{-1})	2.5 ± 0.2	a	74.3	2.5 ± 0.2	a	87.1	5.0 ± 0.5	b	63.6	5.7 ± 0.6	b	70.1
IN (n∘. plant^{-1})	43.0 ± 1.3	c	22.2	26.1 ± 0.7	a	27.3	31.2 ± 0.9	b	19.2	32.9 ± 1.0	b	19.2
IL (cm)	$0.3 \pm 8 \times 10^{-3}$	a	20.1	$0.4 \pm 8.7 \times 10^{-3}$	b	24.0	$0.4 \pm 1.9 \times 10^{-2}$	c	28.8	$0.5 \pm 1.7 \times 10^{-2}$	c	22.5
DLB (mm)	$0.7 \pm 4.2 \times 10^{-2}$	a	43.8	$0.7 \pm 3.8 \times 10^{-2}$	a	54.3	0.9 ± 0.1	b	45.1	0.7 ± 0.1	a	46.7
Leaf-L (cm)	$0.4 \pm 9.4 \times 10^{-3}$	a	17.6	$0.5 \pm 1.1 \times 10^{-2}$	b	21.5	$0.5 \pm 2.0 \times 10^{-2}$	b	26.8	$0.6 \pm 1.3 \times 10^{-2}$	c	13.5
Leaf-W (cm)	$0.1 \pm 3.4 \times 10^{-3}$	a	26.5	$0.1 \pm 4.1 \times 10^{-3}$	b	32.1	$0.1 \pm 6.6 \times 10^{-3}$	b	35.5	$0.1 \pm 5.0 \times 10^{-3}$	c	21.2
Leaf-A (cm^2)	$2.4 \times 10^{-2} \pm 1.6 \times 10^{-3}$	a	49.4	$4.3 \times 10^{-2} \pm 2.2 \times 10^{-3}$	c	53.0	$3.7 \times 10^{-2} \pm 3.2 \times 10^{-3}$	b	57.5	$4.9 \times 10^{-2} \pm 1.6 \times 10^{-3}$	c	20.0
Leaf L/W	4.4 ± 0.1	a	21.0	4.2 ± 0.1	a	18.9	4.3 ± 0.1	a	21.9	4.3 ± 0.2	a	25.5
EO (%)	1.6 ± 0.7	a	99.0	1.0 ± 0.4	a	70.2	2.1 ± 0.5	a	53.2	1.7 ± 0.5	a	72.9

PH: plant height; BN: number of branches per plant; DLB: diameter of longest branch; IN: number of internodes; IL: internode length; Leaf-L: leaf length; Leaf-W: leaf width; Leaf-A: leaf area; Leaf L/W: leaf length/leaf width ratio; EO: essential oil content. * values expressed in percentage. Different letters in the row indicate significant differences ($p < 0.05$).

Regarding foliar parameters, the Tulumba population significantly exceeded the rest of the populations for both leaf length and leaf width, while leaf area was significantly higher than populations of Bialet Massé and Ongamira but did not differ from the population of Río Cuarto.

The essential oil content varied notably among populations, ranging from 1.0% to 2.1% (Table 4), although no significant differences were observed between the populations. The Ongamira population presented the highest essential oil yield, doubling the yield observed in the Río Cuarto population and exceeding the yield of the Bialet Massé and Tulumba populations.

The analysis of the chemical composition of *H. multiflora* essential oil revealed marked phytochemical variability among the four populations studied (Table 5), identifying seven main components with heterogeneous distribution.

Table 5. Chemical composition of the essential oils of the four studied populations of *Hedeoma multiflora*, expressed as a percentage.

	Population			
	Bialet Massé	**Río Cuarto**	**Ongamira**	**Tulumba**
Limonene	0.8	0.6	2.8	-
Menthone	17.0	18.8	63.7	59.1
Isomenthone	-	27.4	2.2	2.10
Isopulegone	0.9	1.2	-	-
Pulegone	75.2	52.0	25.9	36.2
D-Germacrene	1.0	-	-	-
Bicyclogermacrene	-	-	5.3	2.7

Two distinctive chemotypes were identified: one dominated by pulegone, represented by the populations of Bialet Massé (75.2%) and Río Cuarto (52.0%), and another characterized by the predominance of menthone, present in Ongamira (63.7%) and Tulumba (59.1%). Notably, the concentration of these compounds exhibits an inversely proportional pattern among populations. Isomenthone constituted a distinctive marker for Río Cuarto (27.4%), while its presence was marginal in Ongamira and Tulumba (~2%) and absent in Bialet Massé. Among the minor compounds, limonene reached its highest level in Ongamira (2.8%), isopulegone was restricted to Bialet Massé and Río Cuarto, D-germacrene was detected exclusively in Bialet Massé (1.0%), and bicyclogermacrene appeared only in Ongamira (5.3%) and Tulumba (2.7%).

To analyze the magnitude of the variability observed for the variables evaluated, it is necessary to consider the coefficient of variation (CV) of each measured variable. Considering the four populations altogether, the evaluated variables presented different levels of variability. Essential oil content and number of branches per plant showed high coefficients of variation (71.9% and 86.3%, respectively). Meanwhile, leaf length, length and number of internodes, leaf width, plant height, diameter of longest branch, and leaf area showed moderate variability, with coefficients of variation between 24.8% and 53.1%, while leaf length/width ratio showed the lowest coefficient of variation (21.1%).

Analyzing each population individually, the Tulumba population was observed to have the lowest mean CV (33.7%), standing out as the most homogeneous population. Its leaf parameters showed remarkable stability, while essential oil content (CV = 72.9%) and the number of branches (CV = 70.1%) maintained high variability. The Ongamira population exhibited a mean CV of 38.2%, with a relatively uniform distribution of variability among variables. High variability was observed in branching (CV = 63.6%) and leaf area (CV = 57.5%). The Bialet Massé population, meanwhile, presented a mean CV of 40.0%, with high variability in essential oil content (CV = 99.0%) and moderate-to-low variability in leaf parameters. Finally, the Río Cuarto population showed the highest mean CV (42.6%), classifying it as the most heterogeneous population. In this population, the high variability in the number of branches (CV = 87.1%) and branch diameter (CV = 54.3%) stood out.

The analysis of the phenological patterns among the four populations of *H. multiflora* studied revealed highly significant differences in the distribution of phenological states (χ^2 = 82.2, gl = 9, $p < 0.0001$), with a Pearson contingency coefficient of 0.5 indicating an association between population and phenology. As shown in Figure 4, Bialet Massé exhibited a marked dominance of fruiting plants (57.41%), a considerable proportion of individuals in the vegetative state (38.89%), and an absence of flowering specimens. Río Cuarto presented the greatest phenological heterogeneity, with a predominance of plants in the vegetative state (46.30%), followed by plants in the fruiting state (33.33%) and the presence of both flowers/fruits (12.04%) and flowering plants (8.33%), suggesting asynchronous reproductive development. Ongamira, on the other hand, showed an almost

bimodal distribution between plants in the fruiting state (51.11%) and flowering/fruiting state (46.67%), with a total absence of individuals in the vegetative state, evidencing the greatest reproductive synchronization (97.78% of plants in advanced reproductive states). Finally, Tulumba presented the highest proportion of fruiting individuals (72.50%), indicative of a more advanced phenological phase compared to the other populations.

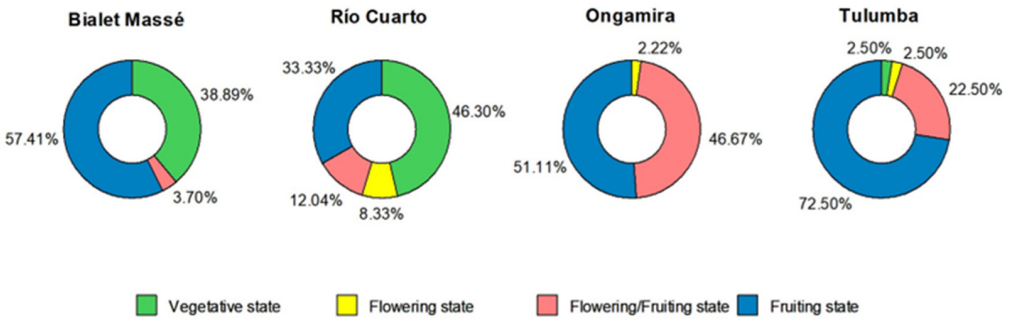

Figure 4. Phenological stages of the individuals of each *Hedeoma multiflora* population, recorded at the sampling time.

Principal Component Analysis (PCA) considering morphological, chemical, and phenological variables showed that 83.9% of the total variability can be explained by the first factorial plane (PC1 and PC2) (Figure 5). At the level of the first principal component (PC1), which explains 52.3% of the observed variability, a clear differentiation is established between the Bialet Massé and Ongamira–Tulumba populations, with the variables with the greatest discriminatory power being pulegone, isopulegone, and the percentage of plants on the vegetative state (negatively associated with PC1) versus the number of branches per plant, internode length, plant height, menthone, bicyclogermacrene, leaf area, and percentage of flowering/fruiting plants (positively associated). At the level of the second principal component (PC2), which explains 31.6% of the variability, the Río Cuarto population is differentiated from the rest of the populations, with isomenthone and the percentage of flowering plants as positive discriminant variables, while the number of internodes per plant, leaf length, leaf wide, Leaf-L/Leaf-W ratio, percentage of fruiting plants and essential oil (EO) content present significant negative loadings.

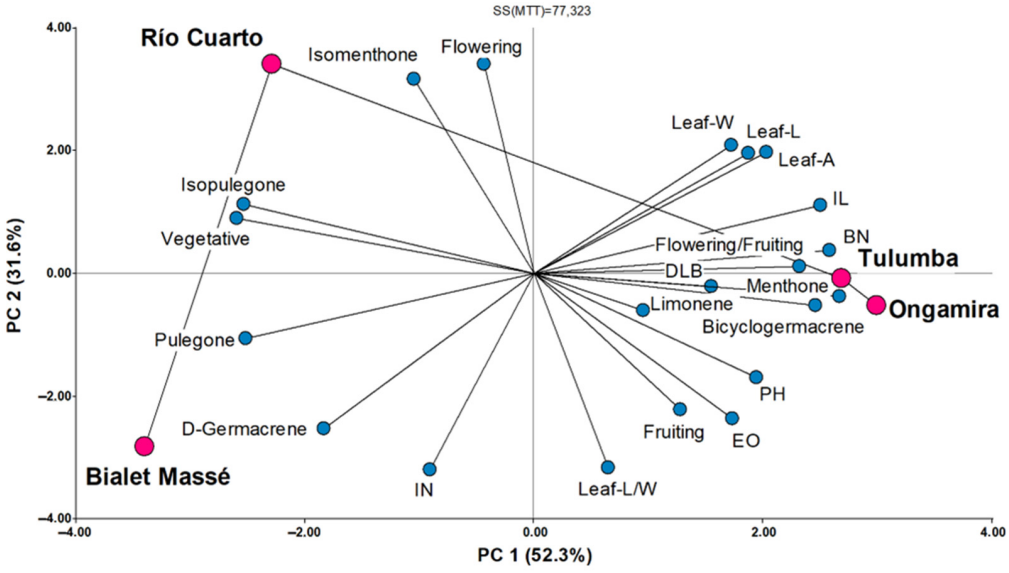

Figure 5. Biplot plane formed by the first two principal components (PC1 and PC2), where the dots represent *Hedeoma multiflora* sampling sites, and the vectors represent morphological, chemical, and

phenological variables. PH: plant height; BN: number of branches per plant; DLB: diameter of longest branch; IN: number of internodes; IL: internode length; Leaf-L: leaf length; Leaf-W: leaf width; Leaf-A: leaf area; Leaf L/W: leaf length/leaf width ratio; EO: essential oil content; Vegetative: plants in a vegetative stage; Flowering: plants in the flowering stage; Flowering/Fruiting: plants in the flowering and fruiting stage; Fruiting: plants in the fruiting stage.

Regarding Generalized Procrustes Analysis (GPA), combining the information obtained for morphological, chemical and phenological variables and the information for soil and climate variables registered in the four sampling sites, the eigenvalues indicate that the first two axes explain 100% of the variability. Figure 6 shows the consensus configuration between the rankings generated by the principal components of both sets of variables. From the ratio between the consensus and the total sum of squares, it can be concluded that there is a 93.8% consensus between the configurations produced by the morphological, chemical, and phenological variables and the soil and climate variables measured in each one of the growing locations.

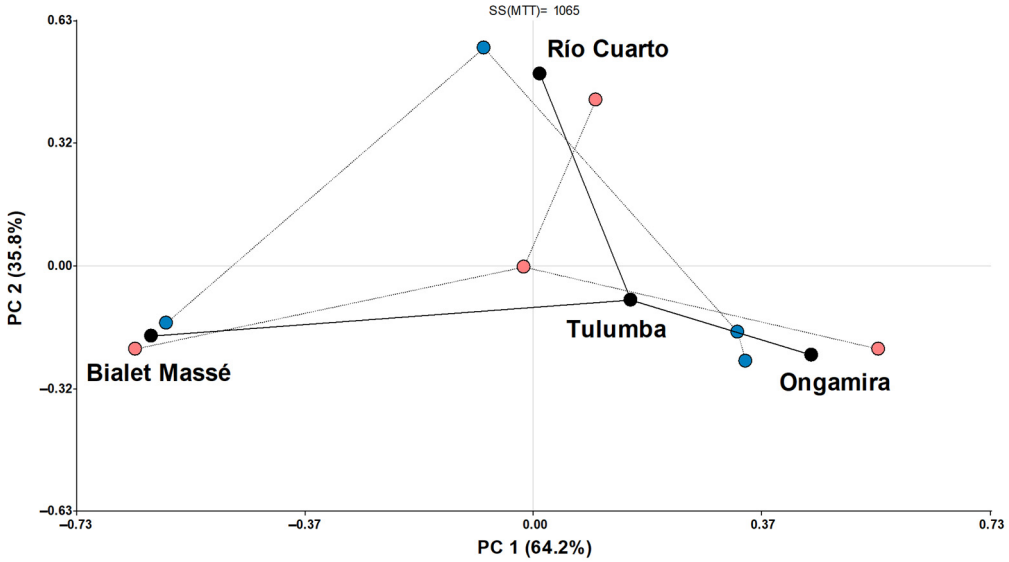

Figure 6. Arrangement of the four *Hedeoma multiflora* populations on the plane formed by the first two axes of a PGA (with minimum tree trail), considering morphological, chemical, and phenological variables and soil and climate variables.

4. Discussion

4.1. Climatic and Edaphic Characterization

Regarding soil parameters, OM content varied considerably across locations, ranging from very low in Ongamira (2%) to very high in Bialet Massé (7.3%). These findings largely align with previously documented OM values for Córdoba soils, which typically fall below 4% [43], consistent with measurements from the Ongamira, Tulumba, and Río Cuarto populations. Only Bialet Massé exhibited notably higher values. Higher soil OM content is associated with enhanced nutrient reserves, cation exchange capacity, water retention, aeration, pollutant degradation capacity, and other quality indicators that influence productivity levels [44]. The same trend was observed when considering the total nitrogen content (tN). Regarding the C:N ratio, Bialet Massé showed the highest value, suggesting a somewhat slower decomposition, while Ongamira showed the lowest ratio, indicating more readily mineralizable organic matter. The Ongamira site demonstrated the least favorable edaphic characteristics among the studied locations, exhibiting the lowest C:N. Decreasing C:N ratios typically indicate soil degradation and increased likelihood of plant nutritional deficiencies [34,45]. Comparatively, the Río Cuarto (2.5%), Tulumba

(3.4%), and Bialet Massé (7.3%) populations displayed similar soil fertility characteristics, with higher OM percentages than Ongamira (2%), and pH values meeting or exceeding the 5.7 threshold—below which chemical fertility issues typically increase [34]. Although Río Cuarto presented the highest EC value, the electrical conductivity measurements indicate non-saline soil conditions at all sites [43,46].

According to provincial soil classification systems, all studied population sites corresponded to class VIIes, designating soils unsuitable for agricultural or livestock applications but valuable for wildlife conservation. This classification includes subclass "e" (erosion), indicating susceptibility to erosive processes, and subclass "s" (root zone limitations), denoting issues with shallow depth, poor moisture retention, salinity/alkalinity, or low fertility [47] (Figure 7).



Figure 7. Sampling sites of wild *Hedeoma multiflora* populations in the province of Córdoba, Argentina: (**a**) Bialet Massé, (**b**) Río Cuarto, (**c**) Ongamira, and (**d**) Tulumba.

The PCA considering soil and climate variables revealed distinct edaphic conditions across the four populations of *H. multiflora*. Bialet Massé soils were characterized by higher organic content. The notably high organic matter content in this population can be attributed to the substantial coverage of decomposing material present in the sampled soil, which is located on private property, while Río Cuarto showed the opposite pattern with lower organic matter. These contrasting soil conditions likely influence the phenotypic expression and adaptation of the different populations, potentially contributing to the species' variability across these locations [6,34].

4.2. Plant Characterization

The results of the morphological characterization of *H. multiflora* populations reveal significant interpopulation variability, consistent with reports by Liébana et al. [16] and Peralta et al. [7,25]. The Tulumba population is distinguished by taller plants (18.51 cm) and larger leaves, which align with observations by Gutiérrez et al. [48], who also reported variations in the growth of this species among different populations. This observed morphological variability falls within the ranges described for the species, which, according to [11] and Barboza et al. [12], presents a height that can vary from 10 to 20 cm in its natural state. However, our findings expand this characterization, recording specimens that exceed these values, thus enriching the taxonomic knowledge of the species.

The essential oil yields observed in the studied populations are within the range of 1.2% and 5.4% except Río Cuarto (1.1%) reported for this species by Koroch et al. [49], Fernández et al. [50], van Baren et al. [15], and Peralta et al. [8]. The absence of statistically significant differences, despite the observed variations, could be attributed to high intrapopulation variability (CV between 53.2% and 99%).

Considering that plants produce essential oils in response to different types of biotic and abiotic stresses, essential oil yield may be associated not only with plant structure but also with environmental growing conditions, which directly impact dry biomass production [51,52].

Regarding the chemical composition of *H. multiflora* essential oil, a particularly relevant finding is the identification of two distinct chemotypes: a chemotype dominated by pulegone (Bialet Massé and Río Cuarto) and another characterized by menthone (Ongamira and Tulumba). This phytochemical differentiation coincides with that described by Fernández et al. [50] and van Baren et al. [15], who also observed variations in the chemical composition of the essential oil of *Hedeoma* plants according to phenological stage and geographical origin.

Similar results were obtained by Nincevic Runjic et al. [53], who studied the medicinal species *Helichrysum italicum*. Their findings support the idea that the physiological and biochemical responses of medicinal and aromatic plants are significantly influenced by environmental growing conditions.

The inversely proportional relationship between pulegone and menthone reflects possible metabolic adaptations to specific environmental conditions, as suggested by Juárez et al. [54]. Furthermore, the variation in minor components such as limonene, isopulegone, and bicyclogermacrene could have ecological implications, possibly associated with defense mechanisms against herbivores or attraction of pollinators, as has been documented in other aromatic species [51].

The high variability observed in essential oil content and the number of branches per plant suggests that these variables exhibit greater phenotypic plasticity, possibly influenced by both genetic and environmental factors. Conversely, the relative stability of leaf length/width ratio indicates possible stricter genetic control or more uniform selective pressure on this variable [6,34].

The stratification of observed variability suggests differential adaptive processes. Populations with greater overall variability (Río Cuarto and Bialet Massé) could inhabit more heterogeneous or altered environments. Conversely, the relative morphological homogeneity of Tulumba suggests greater environmental stability [8].

The morphological heterogeneity is manifested not only between populations but also within them, as demonstrated by the coefficients of variation. The Río Cuarto population presented the greatest intrapopulation variability (mean CV = 42.6%), while Tulumba showed the greatest homogeneity (mean CV = 33.7%). These differences could be attributed to both genetic and environmental factors, a phenomenon also observed by Brunetti et al. [8] in other native aromatic species. Particularly relevant is the high variability observed in the number of branches and essential oil content across all populations, with CVs exceeding 60%, suggesting strong phenotypic plasticity for these traits. In contrast, the leaf length/width ratio showed a low CV (21.1%), indicating greater genetic control over this trait. These observations are consistent with those reported by Brunetti et al. [8] for *Salimaneae integrifolia* (ex Lippia integrifolia), where similar patterns of variability were also found.

The heterogeneous phenological patterns observed among populations may be due to adaptations to the environmental conditions specific to each sampling site or to genetic variations that influence the timing and progression of phenological cycles. These pheno-

logical asynchronies have important implications for in situ conservation and sustainable use of the species, as noted by Elechosa et al. [1] and Gutierrez et al. [48], who recommend adapting harvest periods to the phenological cycle to ensure seed production and natural regeneration. Understanding these patterns is fundamental for developing sustainable collection protocols that preserve long-term population viability [48] and for initiating its domestication and cultivation process [8].

Like the PCA considering soil and climate variables, the PCA considering morphological, chemical, and phenological variables also allowed for the clear differentiation of the four populations of *H. multiflora* evaluated. The GPA combining the information obtained for morphological, chemical, and phenological variables and the information for soil and climate variables indicates that both types of variables allowed establishing differences between the four populations of *H. multiflora* evaluated in a similar manner [34,35,42], and a large portion of the observed phenotypic variability could be explained by adaptations to specific environmental conditions.

The Bialet Massé population, characterized by soils with high organic matter content (7.3%) and greater precipitation (71.2 mm), presented plants with a higher number of internodes, but shorter ones, and a high pulegone content (75.2%). In contrast, Ongamira, with the lowest values of organic matter (2%), showed plants with larger branch diameters and higher essential oil content, with menthone predominating (63.7%). These correlations suggest that edaphic factors, particularly organic matter content and the C:N ratio, have a significant influence on the morphological and phytochemical expression of *H. multiflora*, as documented by Liébana et al. [16] and Peralta et al. [7] for this species, and by Brunetti et al. [8] for other native aromatic plants.

Although this study did not directly evaluate natural regeneration, previous work by [23] and Liébana et al. [16] has documented very low or non-existent natural regeneration rates in several populations of *H. multiflora*, primarily associated with unsustainable harvesting practices and herbivory pressure. This situation is consistent with the classification of the species as threatened according to Martínez et al. [4] and Elechosa et al. [1].

The high interpopulation variability observed in this study underscores the importance of preserving the genetic diversity of this species through conservation strategies that encompass multiple populations. As suggested by Peralta [23], the implementation of germplasm banks and the development of cultivation protocols could complement in situ conservation measures to ensure the long-term survival of this valuable resource.

The results obtained also have important implications for domestication and conservation strategies of *H. multiflora*. The identification of two distinct chemotypes (menthone and pulegone) offers the possibility of selecting material with specific chemical profiles for differentiated commercial applications, as suggested by Peralta et al. [7], Brunetti et al. [8], and Nincevic Runjic et al. [54] for other aromatic species.

The characterization of morphological and chemical variability among populations also provides valuable information for the establishment of ex situ collections representative of the species' genetic diversity. As noted by Peralta [23] and Liébana et al. [16], ex situ conservation through tissue culture and seed banks constitutes a fundamental complementary strategy for threatened species such as *H. multiflora*.

Furthermore, understanding the associations between morphophytochemical characteristics and edaphoclimatic conditions can guide the selection of suitable areas for crop establishment as part of a sustainable use strategy that reduces pressure on natural populations, in line with the recommendations of Elechosa et al. [1] and Peralta et al. [7].

5. Conclusions

This in situ evaluation of four native *H. multiflora* populations in Córdoba province successfully characterized the phenotypic variability in this threatened medicinal species, revealing significant morphological, phenological, and phytochemical differences strongly associated with local edaphoclimatic conditions (93.8% consensus in Generalized Procrustes Analysis).

The morphometric analysis demonstrated clear population differentiation, with Tulumba plants showing the greatest height and leaf dimensions, Ongamira specimens exhibiting the largest branch diameter and highest essential oil content, and Bialet Massé populations distinguished by the highest internode number but the shortest internode length. Two distinct chemotypes were identified, pulegone-dominant (Bialet Massé and Río Cuarto) and menthone-dominant (Ongamira and Tulumba), indicating genetic divergence and/or phenotypic plasticity responses to local ecological pressures.

Phenological assessment revealed significant reproductive asynchrony among populations, with Ongamira showing the greatest synchronization (98% of plants in advanced reproductive states) and Río Cuarto showing the most heterogeneous distribution. High coefficients of variation for essential oil content and branch number (>50%) across all populations indicate considerable phenotypic plasticity in these traits.

Soil organic matter content, ranging from very low in Ongamira (2%) to very high in Bialet Massé (7.3%), significantly influenced morphological and phytochemical expression, with soil parameters explaining 61.7% of total variability in the first principal component.

These findings provide essential baseline information for developing conservation strategies that preserve the genetic diversity of this threatened species and for initiating sustainable cultivation programs. The characterized phenotypic variability constitutes a fundamental step toward the valorization and conservation of this important native phytogenetic resource.

Author Contributions: All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by F.R.T., A.G.C., F.J.d.B. and L.E.T. The first draft of the manuscript was written by L.E.T. and all authors commented on previous versions of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Secretariat of Science and Technology—National University of Córdoba (SECyT-UNC). "Caracterización y evaluación de especies aromáticas y medicinales" (Res. SECyT N° 411-2018) and "Caracterización, domesticación y cultivo de especies aromáticas, medicinales y culinarias" (Res. SECyT N° 258-2023).

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Acknowledgments: The authors are indebted to the Secretariat of Science and Technology—National University of Córdoba (SECyT-UNC) for the fellowship of Florencia R. Turco. The authors also wish to thank Luis Mario Sánchez Salguero, Rodrigo Sánchez Salguero, Rogelio Cuello, Federico Sartori, and Omar Castillo for their help in locating the sampling sites. They also wish to thank Héctor A. Guidobaldi, Martina Guidobaldi, Martín Díaz, María Cecilia Acevedo, María Florencia Melano, Zaida Asis, Lidia Sturm, Alejandra Sánchez Salguero, Rogelio Cuello, and Omar Castillo for their invaluable contribution to this work during the sampling campaigns.

Conflicts of Interest: The authors have no competing interest to declare that are relevant to the content of this article.

Abbreviations

LABSA	Soil and Water Laboratory
ANOVA	Analysis of Variance
PCA	Principal Component Analysis
PC1	First principal component
PC2	Second principal component
GPA	Generalized Procrustes Analysis
Jan-Max	January average maximum temperature
Jan-Min	January average minimum temperature
Jul-Max	July average maximum temperature
Jul-Min	July average minimum temperature
AAT	Annual average temperature
AAP	Average annual precipitation
PH	Plant height
BN	Number of branches per plant
IN	Internode number
IL	Internode length
DLB	Diameter of base longest branch
Leaf-L	Leaf length
Leaf-W	Leaf width
Leaf-A	Leaf area
Leaf L/W	Leaf-L/Leaf-W ratio
EO	Essential oil content
OM	Organic matter
OC	Organic carbon
tN	Total nitrogen
C:N	Ratio: carbon-nitrogen ratio
EC	Electric conductivity
CV	Coefficient of variation

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