**B7. Biodiversity of city Gijón**

**Material and Methods**

During June 2023, five different localities were sampled for seven habitat types resulting in 35 different plots.

Following habitat types have been selected for the present study:

**1. Nature of the first kind = NATURE 1**

REMNANTS of natural/pristine ecosystems

Natural forests around the city (max. 20 km from the city center)

**2. Nature of the second kind = NATURE 2**

REMNANTS of man-made ecosystems resulting from early habitat transformation

agricultural systems – patches of agrarian or silvicultural land uses

Meadows/Pastures in urban and periurban area

**3. Nature of the third kind = NATURE 3**

TRANSFORMED REMNANTS or newly established after habitat destruction

Grassland in urban parks

**4. Nature of the fourth kind = NATURE 4**

EMERGING vegetation after habitat destruction

**4A.** Vacant lots in residential area

**4B**. Transport corridors

**4C.** Vacant lots in industrial area

**4D.** Paved urban areas, e.g. streets, roadside, parking lots, walls

All spontaneous plant species in each plot were recorded and the percentual representation of each species in herbaceous layer approximated. Any unknown plant species were collected and identified in the laboratory. For identification we used key Aizpuru et al. (1999). For nomenclature we followed Euro+Med PlantBase.

Environmental variables of each plot were recorded to test for intercorrelation.

Soil samples for DNA analysis were collected from the four cardinal points of each of the vegetation sampling plots to a depth of 5 cm using a sterile metal core and immediately frozen. Additionally, 500 g of soil samples were collected for chemical composition and other soil characteristics.

DNA analysis – COPIAR DE ALLGENETICS

Limitations of the study.

The recorded flora represents just a subset of possible spontaneous vegetation because this study was carried on during limited time which covers just part of the growing season of the whole vegetation.

There are some other habitats in the city which were not included in the present study, e.g. wetlands, beach, cliffs.

REFERENCES

Aizpuru et al. (1999) Claves ilustradas de la Flora del País Vasco y territorios limítrofes. AIZPURU, I., C. ASEGUINOLAZA, P.M. URIBE-ECHEBARRÍA, P. URRUTIA, I. ZORRAKÍN, eds. (1999). Ed. Servicio Central de Publicaciones del Gobierno Vasco. Vitoria-Gasteiz (España). ISBN: 84-457-1396-5.

Euro+Med 2006+ [continuously updated]: Euro+Med PlantBase - the information resource for Euro-Mediterranean plant diversity. Published at http://www.europlusmed.org [accessed September 20, 2024]

ACKNOWLEDGMENTS

ALLGENETICS for DNA analysis and metabarcoding.

INEA for soil analysis.

Abelardo, María, Sara for fieldwork help.

Sergio y José for laboratory help.

Fundación Biodiversidad for financial support.

Metodología detallada con el orden de pasos a seguir en el campo:

1. **Delimitar el “plot”** (4 m x 4 m) con vegetación homogénea representativa dentro de las parcelas que representan el tipo de ecosistema/naturaleza (al menos de 20 m x 20 m). Apuntar coordenadas y características del plot (cobertura por plantas, inclinación, orientación).

2. **Recoger muestra de suelo para análisis de ADN.** GUARDAR EN FRÍO. TRABAJAR ESTÉRIL

* En cada plot se recoge en cuatro puntos diferentes en condiciones estériles (mascarilla, guantes, tubos y herramientas estériles).
* Escoger cuatro puntos cardinales dentro de la parcela (a 0,5 m hacía el interior desde el borde – esto para que este accesible sin pisar mucho la parcela). Eliminar la vegetación y con una herramienta estéril (tipo core estrecho) recoger la muestra de suelo de cada uno de ellos en un tubo Falcon de 50 ml. Mezclar bien, homogenizar con una lanceta y dividir la muestra en dos tubos Eppendorf de 2 ml. Congelar!!! Uno de los tubos Eppendorf se enviará a extraer el ADN, el otro dos se queda guardados como duplicado. Análisis de ADN (AllGenetics) incluye Extracción de ADN + Preparación de genotecas con 2 parejas de primers diferentes específicos para barcoding (ITS para hongos y 16S para bacterias) + Secuenciación en una plataforma Illumina NovaSeq PE250 (es decir, asumiendo un tamaño de amplicón <450 pb) + Análisis bioinformático estándar, incluyendo el demultiplexado, control de calidad y preprocesado de las muestras, asignación taxonómica, número de lecturas por taxón y generación de curvas de rarefacción.

3. **Realizar el inventario florístico**.

All spontaneous plant species in each plot were recorded and the relative abundance of each species in herbaceous layer approximated.

4. **Medir los rasgos de plantas (plant traits + seed traits).**

* Para las especies cuya abundancia relativa sea mayor de 20% se estudiarán los traits. Se mide la altura de planta en 10 individuos (hasta la última hoja y hasta la última flor en el campo). Se recogerán hojas maduras sin daños visibles de unos 10 especímenes en bolsas de plástico para el estudio SLA (escanear y pesar en fresco y en seco).
* Si alguna de estas las plantas ya tiene frutos, estos se recogen para el posterior estudio de rasgos y germinación de semillas. Si no tiene fruto desarrollado, habrá que volver a recoger los frutos más adelante

5. **Tomar muestras de suelo para análisis físico-químico.** (bolsa de 1 kg aprox.)

* Eliminar la hojarrasca y la vegetación superficial. Coger muestra en 5 puntos diferentes (central + 4 puntos cardinales) y mezclar en una bolsa de plástico. Homogenizar, dejar secar y enviar a analizar a INEA Valladolid. Análisis completo (pH, textura, conductividad eléctrica, materia orgánica, nitrógeno total, relación C/N, caliza activa, carbonatos totales, fósforo asimilable, K, Ca, Mg, Na) + densidad aparente (bulk density), metales pesados (Zn, Cr, Cu, Cd, Hg, Pb, Ni)

**Main ideas “urban vegetation” from bibliography**

**Cities**

1. highly fragmented landscapes

2. mosaics of heterogeneous ecosystems (in terms of size, fragmentation, population density and land use)

3. subject to different degrees of human interference, leading to a stepwise transformation of pristine ecosystems to novel urban ecosystems

4. comprised of a patchwork of paved and unpaved spaces, built and vacant land, and newly developed and obsolescent and/or abandoned buildings and infrastructure

5. contains a multitude of vacant lots, railway sidings, utility easements, corridors between buildings and canal sides that are often overgrown with spontaneous vegetation, which are not coherently managed, and which seem to occupy an uncertain, interstitial niche in the urban matrix

Cities are comprised of a patchwork of paved and unpaved spaces, built and vacant land, and newly developed and obsolescent and/or abandoned buildings and infrastructure. They are mosaics of heterogeneous ecosystems (in terms of size, fragmentation, population density and land use) which are subject to different degrees of human interference, leading to a stepwise transformation of pristine ecosystems to novel urban ecosystems (Kowarik, 2005).

The “Four Nature approach” narrows down the variety of transformational stages in urban settings to four major types that can supply wilderness in urban region (Kowarik 1992, 2005). Each of the four types relates to nature in general but results from different trajectories in human-nature interactions:

Spontaneous vegetation appears in different habitat types in the urban matrix,. Multitude of vacant lots, railway sidings, utility easements, corridors between buildings and canal sides that are often overgrown with spontaneous vegetation, which are not coherently managed, and which seem to occupy an uncertain, interstitial niche in the urban matrix. The spontaneous herbs could provide the very interesting pool of species for ecological restoration of the urban area. Their ecological value has been clear since many years because their contribution to the ecosystem services in urban areas. Although their aesthetic value has been highlighted repeatedly, their acceptance by the residents in urban areas has been questioned. Many residents associate the presence of spontaneous plants with the neglect in maintenance by public (municipal) management service. Spontaneous native urban vegetation has commonly been described as demonstrating resilience (Ignatieva et al., 2000) and exhibiting adaptations to human disturbance (Lundholm and Marlin, 2006; Sukopp, 2004).

Urban species pool is comprised of native species present before the urban development, native species developed because the new urban ecosystem and introduced species by human activity. However, just some of them can adapt to urban environment.

IGS (informal green space) consists of any urban space with a history of strong anthropogenic disturbance that is covered at least partly with non-remnant, spontaneous vegetation (Del Tredici, 2010).