

Gaps and biases in the ecological evidence underlying global biodiversity impact assessments

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Abstract

1. Halting global biodiversity loss requires methods that link biodiversity impacts to both their direct drivers and the human activities that generate them. Biodiversity footprint assessment has emerged as a key framework for establishing these connections, resulting in the rapid development of diverse methods and applications. Central to these approaches are characterisation factors (CFs), which translate environmental pressures, such as land use, into quantified biodiversity impacts. CFs are based on empirical ecological studies that vary in taxonomic, geographic and methodological scope, potentially introducing uncertainty, bias and limited representativeness into biodiversity footprint assessments.

2. We systematically examined the empirical foundations of land-use pressure–impact relationships underpinning CFs in four widely used global biodiversity impact assessment methods. We traced the original empirical studies on which the CFs were based, compared overlap among the methods' publication bases, and assessed their taxonomic, geographic representativeness. We also compared these studies with a recent global database of human impacts on biodiversity and identified priority ecoregions for future research by combining evidence gaps with the occurrence of Key Biodiversity Areas and high anthropogenic land-use pressure.

3. The four methods relied on markedly different but partly overlapping evidence bases. Across methods, the underlying evidence showed pronounced taxonomic and geographic biases. Vertebrates and vascular plants were generally overrepresented, whereas invertebrates, amphibians, reptiles, fungi and many other groups were poorly represented or absent, although the extent of these gaps varied among methods. Evidence was concentrated in (sub)tropical moist broadleaf forests and in Asia, Europe and the Americas, with substantial gaps in Africa, Australia and Oceania.

4. This uneven representation has important implications for the interpretation, robustness and global comparability of biodiversity footprint assessments. Building on our findings, we identify priority regions for future research and highlight opportunities to strengthen biodiversity impact assessment by incorporating more balanced ecological evidence into CF development and promoting structured, assessment-relevant biodiversity data collection. Greater transparency about the empirical foundations, representativeness and limitations of CFs is also essential to support more robust biodiversity footprint assessments and better-informed strategies for mitigating biodiversity loss.

Keywords: biodiversity loss; biodiversity footprints; pressure-impact relationship; characterisation factors; land use; life cycle assessment; impact assessment

1. Introduction

Global biodiversity loss continues at an alarming rate. Wildlife populations have declined and current extinction rates exceed natural background levels by a wide margin (IUCN, 2026; Ceballos et al., 2015; Pimm et al., 2014). To halt and reverse this trend, international and regional biodiversity policies and frameworks, such as the Kunming-Montreal Global Biodiversity Framework (CBD, 2022) and the European Commission's Biodiversity Strategy for 2030 (European Commission, 2020), have been agreed upon. These commitments stress the need to integrate considerations of biodiversity with public and private sector decision-making. To operationalise these aims, it is essential to assess and measure drivers of biodiversity loss, such as land use and other resource exploitation, and link them to the underlying consumption and production patterns and other human activities. To this end, scientifically robust and transparent approaches and tools for assessing anthropogenic impact on biodiversity are of crucial importance (IPBES, 2026; Damiani et al., 2023).

Biodiversity footprint assessment has become a widely used approach by businesses and other organisations to assess their biodiversity impacts, including those generated via value chains. The results can further be used to evaluate compliance with emerging disclosure frameworks (Kundi et al., 2026) and design effective impact mitigation strategies (Taylor et al., 2023). Multiple methods have been developed to quantify the biodiversity impacts along the value chain, including process-Life Cycle Analysis (LCA), Input-Output (IO)-LCA (e.g. Ruokamo et al., 2023; Liu et al., 2026), and physical material flow based assessments (e.g., Bidoglio et al., 2026). Of these, the first one often focuses on assessing the impacts of a specific product, process or an organization, and the latter two on analysing impacts at a macro scale, i.e. at the national, regional, or global level.

A common denominator for these approaches is the use of characterisation factors (CFs), sometimes referred to as impact factors, to translate environmental pressures, such as land use or greenhouse gas emissions, into impacts on biodiversity (i.e. pressure-impact relationships). The CFs are derived from a characterisation model to convert a specific inventory flow, e.g. hectares of land used or kilograms of greenhouse gas emissions, to a unit of the biodiversity impact, most commonly the potentially disappeared fraction of species (PDF) or the loss of mean species abundance (MSA) (Damiani et al., 2023). Hence, CFs are a fundamental part of measuring and estimating potential anthropogenic impacts on biodiversity.

The empirically grounded ecological field data describing the state of biodiversity and its responses to different pressures provides the foundation on which CF components are established. Yet, several limitations in the underlying data that the CFs are based upon, such as variation in the taxonomic and geographic representativeness, can introduce uncertainty and bias in the resulting CFs. This, in turn, risks misrepresenting real-world impacts, which are highly context-dependent (Bromwich et al., 2025) and would require a broad representation of taxa and habitats to reflect these contexts. Differences in reference periods and reference areas can further contribute to variation across CFs (Soimakallio et al., 2025; Damiani et al., 2023). Moreover, the spatial specificity and

resolution of both the underlying datasets and the derived CFs may differ between approaches. To what extent such biases exist in the multiple impact assessment approaches has, however, not been analysed.

Several studies have shown that different methodological approaches based on varyingly constructed CFs can lead to substantial discrepancies between individual impact assessment results, even when identical inventory flow data have been applied (Martínez-Ramón et al., 2025; Sanyé-Mengual et al., 2022; Bromwich et al., 2025). In some cases, the choice of method can even alter which impact category (e.g. land use, water use, or climate change) has the largest impact (Martínez-Ramón et al., 2025). Understanding the reasons behind this variability is critical, as it may consequently lead to the adoption of potentially misdirected mitigation strategies (Martínez-Ramón et al., 2025). Inconsistent, and even misleading, results and the lack of methodological transparency can erode the trust and societal acceptance of biodiversity footprint assessment, highlighting the importance of understanding the basis on which impact assessments stand.

Therefore, improved understanding of the differences among biodiversity footprint assessment methods can help the practitioner understand the types of questions to which specific CFs can be applied coherently. In addition, transparent information about the empirical foundation of CFs is essential for supporting robust interpretation of biodiversity footprint assessments and ensuring comparability across approaches.

In this study, we ask two main questions: (1) What empirical evidence underpins the pressure–impact relationships used in CFs in global biodiversity footprint assessment methods, and to what extent do these evidence bases overlap? and (2) What are the main sources of uncertainty and bias, such as uneven taxonomic or geographic representativeness, in these data? To answer our questions, we analysed four widely used biodiversity impact assessment methods. We focused on a single pressure, land use, which is quantified in all four studied methods. Land use is the primary driver of global biodiversity loss (IPBES 2019; Jaureguiberry et al., 2022) and is therefore included in all major biodiversity impact assessment methods (Crenna et al., 2020; Damiani et al., 2023). We reviewed method descriptions, identified the original empirical studies used to establish the pressure–impact relationships used in them, and compared the taxonomic and geographic coverage of the data. Furthermore, to explore potential and relevant evidence not yet incorporated into these methods to relieve some of the biases and strengthen the knowledge base, we also compared the data used in the four methods to a database on human impacts on biodiversity (Keck et al., 2025). Building on these insights, we discuss how well widely used biodiversity impact assessment methods reflect impacts on global biodiversity and provide recommendations on how to improve their transparency, representativeness, and robustness.

2. Material and methods

2.1. Selection of biodiversity impact methods

We selected biodiversity impact methods that represent widely applied, comprehensive, and global approaches currently available for quantifying biodiversity impacts: ReCiPe 2016 (Huijbregts et al., 2016; Huijbregts et al., 2017), Impact World+ (IW+) (Bulle et al., 2019), Global Life Cycle Impact Assessment Method phase 3 (GLAM3) developed within the Life Cycle Initiative hosted at United Nations Environment Programme (UNEP) (Verones et al., 2026a), and the intactness-based biodiversity impact factors (IBIF) dataset (Schipper et al., 2025) derived from Global biodiversity model for policy support (GLOBIO4) (Schipper et al., 2020). The CFs from these methods are widely applied in operational LCA practices (Crenna et al., 2020; Damiani et al., 2023). ReCiPe and IW+ are additionally available as integrated methods in LCA software. All four approaches are increasingly used in IO-LCA and other approaches, including in environmentally extended multi-regional input–output (EE-MRIO) analyses (e.g. Ruokamo et al., 2023; El Geneidy et al. 2026), and physical material flow assessments (e.g. Bidoglio et al., 2026). Together, these methods cover a broad range of biodiversity-relevant pressures. However, to allow comparability of the empirical knowledge base underlying pressure–impact relationships, we only focused on one pressure, land use, which is included in all four methods.

2.2. Review of the knowledge base for land use pressure-impact relationship

All methods rely on diverse data inputs to derive CFs, such as species' geographic ranges, habitat classifications, z-values describing the relationship between species richness and area, and dispersal distances. However, our analysis focuses on the pressure–impact relationships and the empirical ecological studies underpinning them.

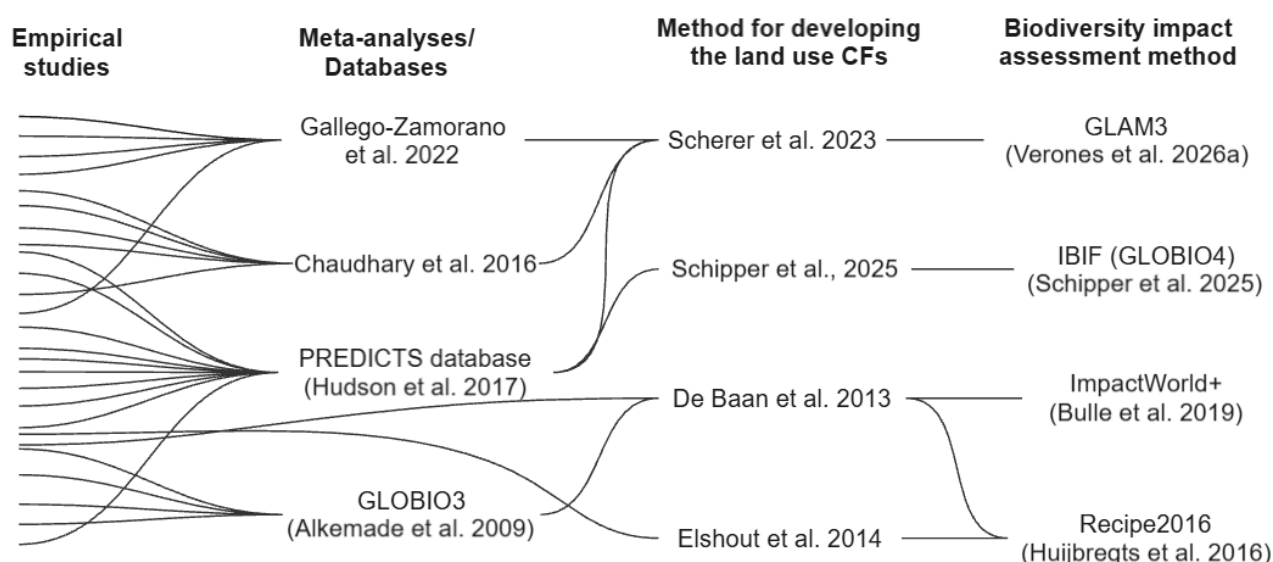
In the underlying evidence base of all four methods, biodiversity impacts are quantified using a space-for-time substitution approach. This approach estimates biodiversity change by comparing species richness or abundance in a given land-use category to that observed in a reference ecosystem located elsewhere, thereby approximating the baseline state of biodiversity (IPBES, 2018). The reference state is typically derived from observational data from current (semi-)natural habitats within the same ecoregion (Scherer et al., 2023; de Baan et al., 2013) or biome (Elshout et al., 2014). In many tropical regions, where historical human influence has been relatively limited, these reference sites often closely represent undisturbed ecosystems. In contrast, in regions with a long history of anthropogenic disturbance, such as Europe, truly undisturbed areas are scarce. Consequently, available reference sites are better described as (semi)natural rather than pristine, as they do not fully reflect pre-human ecological conditions (de Baan et al., 2013).

The land use impacts on biodiversity across the different methods are captured using different approaches, which we briefly outline here. More detailed descriptions are provided in the Results section in Table 1 and in the Supplementary Information.

2.2.1. ReCiPe 2016

ReCiPe produces midpoint CFs (expressed in $\text{m}^2\cdot\text{yr}$ annual crop equivalents) and endpoint CFs (species/ m^2) analysing species richness (number of species) under

187 anthropogenic land use types (annual crops, permanent crops, mosaic agriculture,
 188 forestry, urban land, pasture) compared to reference natural land cover (Huijbregts et al.,
 189 2016). Relative species loss is calculated by comparing empirical observations of local
 190 species richness using a linear relationship (described by Köllner and Scholz, 2007) and is
 191 based on two meta-analyses (de Baan et al. (2013) and Elshout et al. (2014) (Fig. 1).
 192 ReCiPe provides global level CFs.
 193



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 195
 196 **Figure 1.** Empirical studies, meta-analyses and databases included in the development of
 197 characterisation factors (CFs) for land use pressure- impact relationships in the four methods
 198 assessed in this study. Links drawn from empirical studies to meta-analyses and databases are
 199 illustrative; see Fig. 2 for the proportion of empirical studies shared between each method pair.
 200

201 2.2.2. IMPACT World+

202
 203 IW+ uses land use CFs from a meta-analysis (de Baan et al. 2013) in which two data
 204 sources were combined to quantify species richness in different land use types: the
 205 GLOBIO3 database (Alkemade et al., 2009) and national monitoring data from Switzerland
 206 (BDM 2004; Bulle et al., 2019) (Fig. 1). The number of species found in a disturbed habitat
 207 is compared to that of a habitat in (semi)natural reference state in the same biome.
 208 Contrary to ReCiPe, IW+ provides spatially differentiated CFs at the biome and country
 209 level.
 210

211 2.2.3. Intactness-based biodiversity impact factors (IBIF)

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 213 IBIF provides land-use CFs both as aggregated impact factors, including habitat loss,
 214 fragmentation, and disturbance, and as disaggregated factors for each of these impact
 215 pathways. To ensure comparability with the other methods assessed, we focus here only
 216 on the disaggregated habitat-loss CFs. These CFs were developed using the mean
 217 species abundance (MSA) metric and GLOBIO4 biodiversity model (Schipper et al., 2020;
 218 Schipper et al., 2025). MSA represents the mean abundance of originally occurring
 219 species relative to their abundance in undisturbed reference conditions. For IBIF, empirical

MSA data used in GLOBIO4 were pooled across land-use intensity levels and used to fit impact relationships for four aggregated land-use types: cropland, pasture, forest plantations, and urban land, for plants and warm-blooded vertebrates (Schipper et al., 2025). The underlying empirical data were compiled from the PREDICTS database (Hudson et al., 2017; Hudson et al., 2023) (Fig. 1). These relationships were combined with spatial land-use data in GLOBIO4 to quantify MSA loss at a resolution of 10 arc-seconds. Country-level habitat-loss CFs for IBIF were then calculated as the area-integrated MSA loss associated with each land-use category divided by the area occupied by that land-use category in the country. IBIF thus provides spatially differentiated CFs at the country level.

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231 2.2.4. GLAM3

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Land use impact accounting in GLAM3 is based on a method developed by Scherer et al. (2023) which has also been adopted in LC-IMPACT v2.0 (Verones et al., 2026b). The method builds on the species-habitat relationship introduced by Kuipers et al. (2021). To obtain the CFs, three levels of management intensity (minimal, light, and intense) as well as the impact from land occupation, transformation, and the effect of fragmentation on species richness are considered. The key component defining the pressure-impact relationship is the relative species loss (RSL) defined as the species richness due to land occupation and land transformation compared with species richness of a habitat in natural state. GLAM3 provides spatially differentiated CFs at the ecoregion and country level. The change in local plant species richness due to converting natural areas to a specific land use type (cropland, pasture, plantations, managed forests, or urban land) and intensity level (minimal, light, or intense) for a species group was based on a meta-analysis (Gallego-Zamorano et al. 2022). The relative species richness for vertebrates was derived from the PREDICTS database (Hudson et al., 2017) for all land use types except for managed forests, for which data from Chaudhary et al. (2016) was used (Fig.1).

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249 2.3. Analysis of the knowledge base

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We reviewed method descriptions, meta-analyses, and databases used for defining CFs in each method. This allowed us to identify the original empirical studies used to establish pressure-impact relationships between land use and biodiversity impacts in each method. From the method descriptions and meta-analyses, we extracted information on the studies, including the bibliographic reference, taxonomic groups assessed, study and reference location, including the coordinates of the study area, information about the biome and the sampling period whenever this information was available. When the data for biomes was not reported in the datasets, we overlaid the coordinates with the biome maps from Olson et al. (2001). For both ReCiPe and IW+ methods, data from de Baan et al. (2013) had been used and augmented with observations from the Swiss national biodiversity monitoring programme (BDM, 2004). As coordinates were not reported for the BDM observations, all corresponding data points were assigned the coordinates of central Switzerland (46.81° N, 8.23° E). These coordinates were used only for visualisation purposes and for identifying the ecoregions with no data included.

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For the analysis, the data was analysed at two levels of aggregation:

- 1) Overlap of publications. We quantified the overlap among the knowledge bases of the four methods by comparing their underlying publications. For each reference, information on authors, publication year, and publication title was collected where available (see Supplementary Data). The datasets from de Baan et al. (2013), which underpin ReCiPe and IW+, and Elshout et al. (2014), which underpins ReCiPe, included only abbreviated references containing author names and publication years without publication titles. Consequently, different publications by the same author(s) in the same year could not be distinguished, and the comparison of knowledge bases was based solely on author names and publication year, resulting in potential misrepresentation of the overlap. In contrast, the datasets underlying GLAM3 and IBIF also included publication titles, allowing a more precise comparison of the underlying publications.
- 2) Representativeness of the studies. To assess taxonomic, regional and biome-level representativeness, we standardised the datasets because the source databases and meta-analyses reported data at varying levels of aggregation, ranging from individual observations to site- or publication-level summaries. Data points were aggregated by year, taxonomic group, and biome, with each unique combination of these three variables considered a distinct study. Thus, a single publication could contribute multiple studies if it contained data from more than one year, taxonomic group, or biome. For the spatial analyses, however, we retained all available individual coordinates rather than using these aggregated studies. The full coordinate-level datasets were used to map the distribution of data points in Figure 3 and to identify priority ecoregions lacking data points in Figure 5.

In addition, we compared the publications underpinning the four methods with the database recently compiled by Keck et al. (2025). This database includes 2133 publications covering data from 97783 impacted and reference sites, thus providing a dataset of 3667 independent comparisons of biodiversity impacts across multiple taxonomic groups and habitats including the impacts of multiple pressures on biodiversity. Of this dataset, 444 publications focus on habitat change in terrestrial biomes across a wide range of taxa and is thus comparable to the studies on land use impact used in the four methods studied here.

To identify potential taxonomic biases in the biodiversity impact methods, we compared the share of different taxonomic groups in the datasets used for developing the CFs to the share of estimated described species per taxonomic group (based on information from the IUCN Red List (2026)). To evaluate geographic representativeness, we conducted two analyses. First, to assess the representation of the different biomes in the data used for developing the CFs, we harmonised biome classifications according to Olson et al. (2001) and compared the share of studies from different biomes with the global extent of them. Second, we identified priority regions for future research by mapping the geographic locations from which data had been derived to develop the CFs and overlaid them with spatial datasets on Key Biodiversity Areas (BirdLife International, 2026) (Fig S1) and anthropogenic land use, including cropland, pasture, grassland, and urban land cover, from HYDE 3.5 (Klein Goldewijk, 2025) (Fig S2). We identified areas which fulfilled the following three criteria: 1) they were not represented in the underlying datasets; 2) they contained Key Biodiversity Areas (KBAs); and 3) they were subject to the highest

anthropogenic land-use pressure quantified as the proportion of high-intensity land-use pixels (>50 km² per grid cell) relative to the total number of pixels within each ecoregion.

3. Results

3.1. Evidence base underlying pressure-impact relationships

Three of the methods, GLAM3, ReCiPe and IW+, based their land use pressure-impact estimation on relative species richness, which in turn was based on multiple case study publications (Table 1). The CFs of GLAM3 relied on the broadest set, including altogether 699 publications from 1986-2019. The CFs of ReCiPe, in turn, relied on 394 publications from 1975-2012, while CFs of IW+ were based on 233 publications from 1977-2009 (Table 1). IBIF, which builds on the GLOBIO4 model, developed its pressure-impact relationships on species abundance ratios in disturbed habitat versus reference sites. These relationships were based on 78 publications from 1996-2015 with multiple sampling sites in each (Schipper et al., 2025), thus constituting the smallest set of publications among all four scrutinised methods in this study (Table 1).

The four methods differed not only in spatial resolution of the developed CFs but also in the scale of geographic information for CF derivation (Table 1). ReCiPe and IW+ were based on biome-level comparisons of pressure-impact relationships. CFs in IBIF were based on the GLOBIO4 model, where the pressure-impact relationships were quantified at the global level and then applied to the spatially explicit land use data. In GLAM3, pressure-impact relationships were quantified at the ecoregion scale for vertebrates across broad land-use classes, while global-scale relationships were used for plants and for vertebrate responses to different levels of land-use intensity.

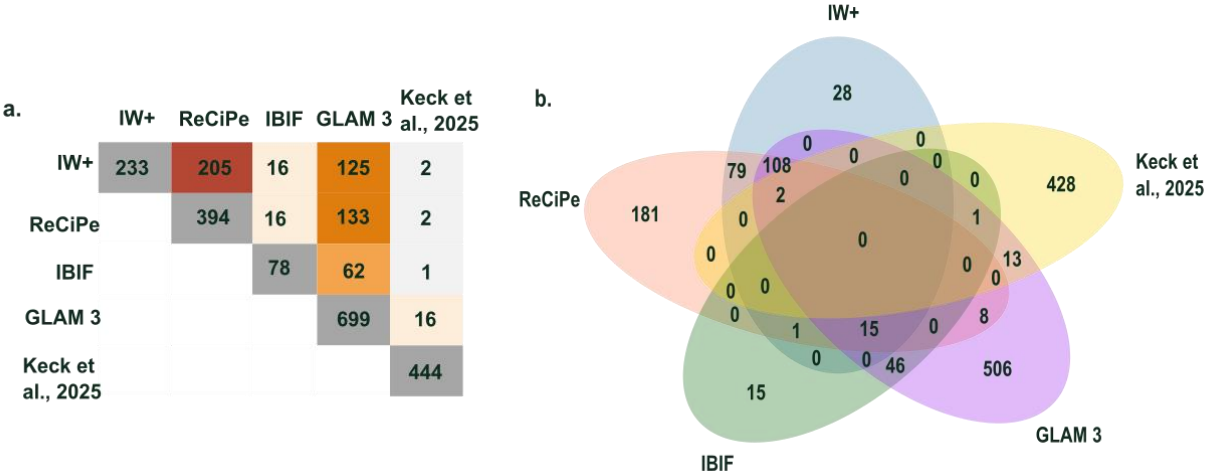
Table 1. Summary of data used to estimate pressure-impact relationships for land use impact in the four methods assessed in this study.

	ReCiPe 2016	IMPACT World+	IBIF (GLOBIO4)	GLAM3
Unit of impacts	species·year	PDF·m ² ·yr	MSA-loss·km ² ·yr	PDF·yr
Number of publications on which land use pressure-impact relationship was based.	394	233	78	699
Temporal span of publication years	1975-2012	1977-2009	1996-2015	1986-2019
Definition of reference area	Potential natural vegetation, describing the expected state of mature	Potential natural vegetation, based on monitoring data from current (semi)natural	Sites categorised as 'Primary vegetation – minimal use'.	The "hypothetical biotic potential" reflecting the current natural habitat found

	vegetation that would develop if all human activities ceased. Approximated using monitoring data from current (semi)natural habitats in the same biome or ecoregion.	habitats in the same ecoregion.		elsewhere within the same ecoregion.
Years between sampling of the reference and disturbed sites	Not available	Not available	0-12 (mean 0.18)	0-22 (mean 0.34)
Taxonomic groups covered	Plants, vertebrates (mammals and birds), and invertebrates (mainly arthropods).	Plants, vertebrates (mammals and birds), and invertebrates (mainly arthropods)	Plants and warm-blooded vertebrates (mammals and birds)	Vascular plants, amphibians, birds, mammals, and reptiles. The underlying data also includes data for invertebrates.
Spatial differentiation of the CFs	Global	Biome and country-level	Country-level	755 ecoregions / country-level
Spatial differentiation of pressure-impact relationship	Biome-level	Biome-level	Global	Ecoregion-specific for vertebrates and broad land use classes. Global for plants and for vertebrate responses to land-use intensity-levels.
Biomes covered out of the 14 biomes defined by Olson et al. 2001	11	8	12	14
Key references	Huijbregts et al., 2016 & 2017; de Baan et al. 2013; Elshout et al. 2014	Bulle et al., 2019; de Baan et al. 2013	Schipper et al., 2025; Schipper et al., 2020	Scherer et al., 2023

3.2. Overlaps in background data

346 Several of the assessed methods relied on overlapping data sources (Fig. 1). For example,
 347 the PREDICTS database provides the bulk of data for both the GLAM3 and IBIF methods.
 348 Similarly, the methodological framework and publications behind the CFs in a meta-
 349 analysis of de Baan et al. (2013) underpin both IW+ and ReCiPe (Fig. 1).
 350
 351 This shared data foundation was reflected as a substantial overlap between the methods
 352 when their underpinning publications were compared (Fig. 2). IW+ and ReCiPe showed
 353 the highest degree of commonality, with 205 shared publications, corresponding to nearly
 354 90% of the publications included in IW+. Also, IBIF and GLAM3 showed a strong overlap,
 355 sharing 62 publications, with nearly 80% of publications used in IBIF also included in
 356 GLAM3, despite the different units of biodiversity loss used (MSA vs. PDF). IBIF had little
 357 overlap with IW+ and ReCiPe (<20 shared publications).
 358
 359 Our comparison of the publications underpinning the four methods with those describing
 360 human impacts on biodiversity from Keck et al. (2025), showed only a minimal overlap,
 361 with just 1-16 shared publications. In total, 428 publications included in Keck et al. (2025)
 362 were not incorporated into any of the four methods (Fig 2).



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 365 **Figure 2.** Overlap among the publications underpinning IMPACT World+ (IW+), ReCiPe 2016, IBIF
 366 (GLOBIO4), GLAM3, and the terrestrial habitat change studies included in Keck et al. (2025). (a)
 367 Matrix of pairwise overlaps, showing the number of publications shared between each dataset. (b)
 368 Venn diagram illustrating all possible overlaps between the five sets of publications.
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371 3.4. Taxonomic and geographic representativeness 372

373 All assessed methods showed strong taxonomic and geographic biases. Overall, we found
 374 uneven global coverage and a dominance of certain taxa, with the biases additionally
 375 differing between the four methods. Most methods covered well-studied groups such as
 376 birds, mammals, and vascular plants. The largest differences were found between IBIF
 377 and GLAM3, for which the first covered the smallest set of data only for birds, mammals
 378 and plants, while the latter included the most extensive dataset covering, in addition to
 379 birds, mammals and plants, also reptiles and amphibians (Fig. 3, Table S1). GLAM3,
 380 ReCiPe, and IW+ also included invertebrates, mainly arthropods. In GLAM3, nearly 400

381 invertebrate publications were incorporated, however these were applied to supplement
382 data for developing CFs for vertebrates and not to develop invertebrate-specific CFs
383 (Scherer et al., 2023).

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385 When compared to global species richness per taxonomic group (IUCN, 2026),
386 vertebrates and vascular plants were overrepresented in all assessed methods (Fig. 3,
387 Table S1). Invertebrates, which account for 69% of all described species, and in particular
388 non-insect arthropods and other invertebrates, were underrepresented, and entirely absent
389 from IBIF. Only GLAM3 incorporated multiple studies for reptiles and amphibians. None of
390 the methods covered fungi and protists or other groups, such as bacteria, for estimating
391 their CFs (Table S1).

392
393 Regarding geographic representativeness, Asia, Europe, and North and South America
394 dominated all four methods, especially GLAM3 and ReCiPe, whereas Africa, Australia,
395 and Oceania were less represented (Fig. 3).

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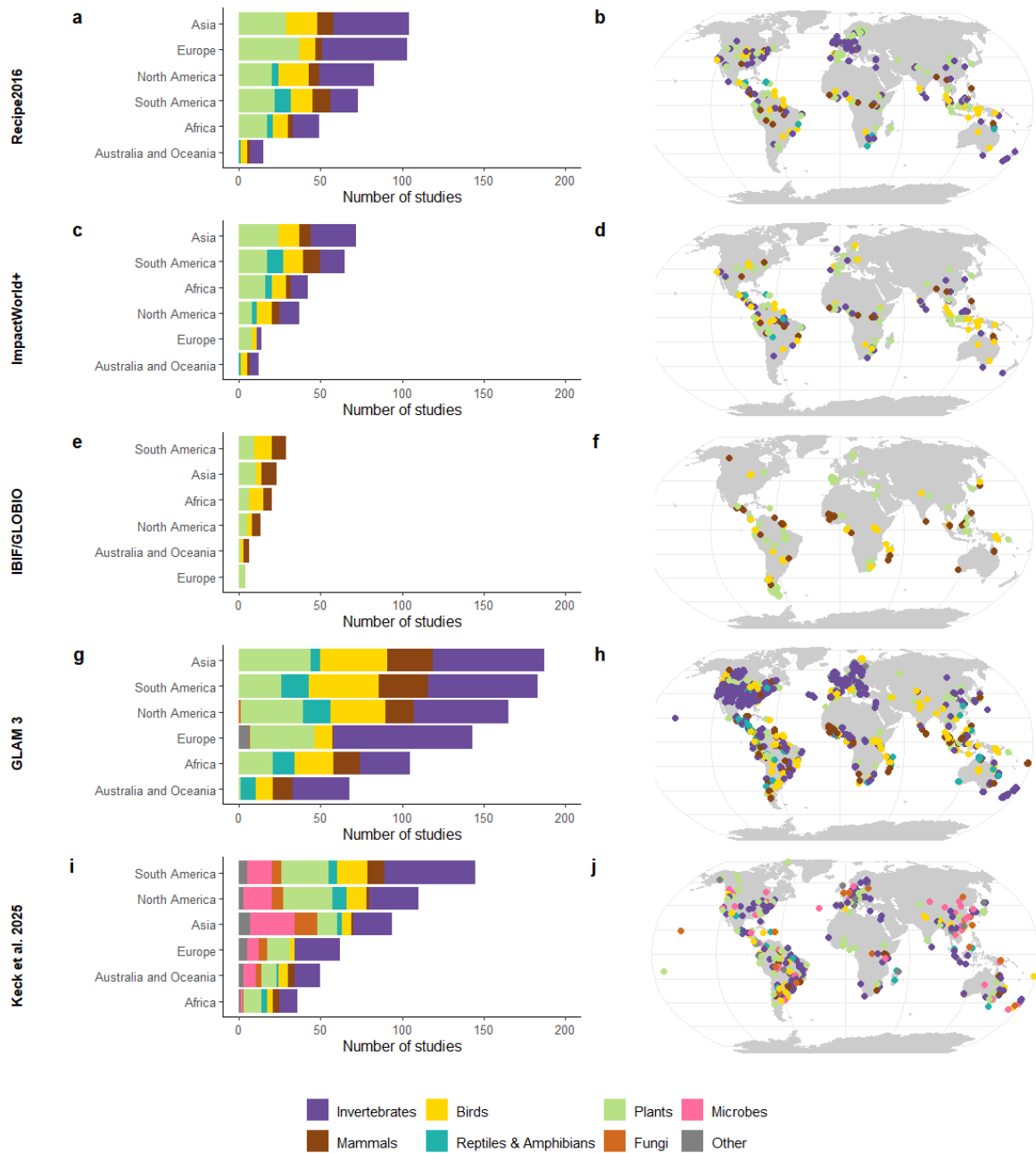
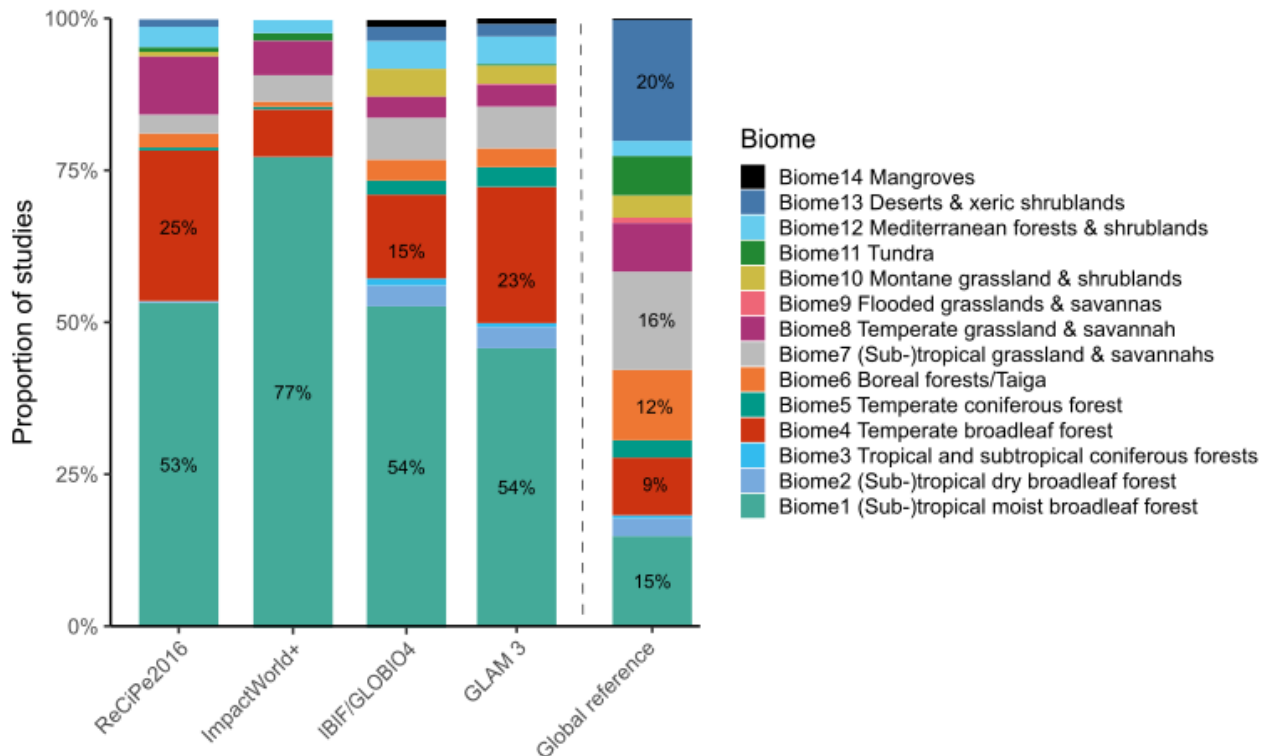


Figure 3. Geographic and taxonomic representativeness of studies underpinning land-use pressure–impact relationships across the four biodiversity impact assessment methods, represented per panel rows: a) and b) ReCiPe 2016; c) and d) IMPACT World+ (IW+); e) and f) IBIF (GLOBIO4); g) and h) GLAM3; and i) and j) studies assessing habitat change in terrestrial biomes from Keck et al., (2025). Panels in the left-hand column show the number of studies per region, while panels in the right-hand column show the geographic distribution of the studies. To ensure comparability among underlying datasets, individual observations were aggregated by year, biome and taxonomic group. Consequently, a single publication contributing data for multiple years, taxonomic groups or biomes is represented as multiple studies in the figure.

Across all methods, studies underpinning the CFs were strongly concentrated in (sub)tropical moist broadleaf forests. Despite this biome only representing 15% of global biome extent, it accounted for 77% of studies in IW+ and more than half of those included in ReCiPe, IBIF, and GLAM3 (Fig. 4). Temperate broadleaf forests were also relatively well represented across all studies. In contrast, several biomes, including boreal forests,

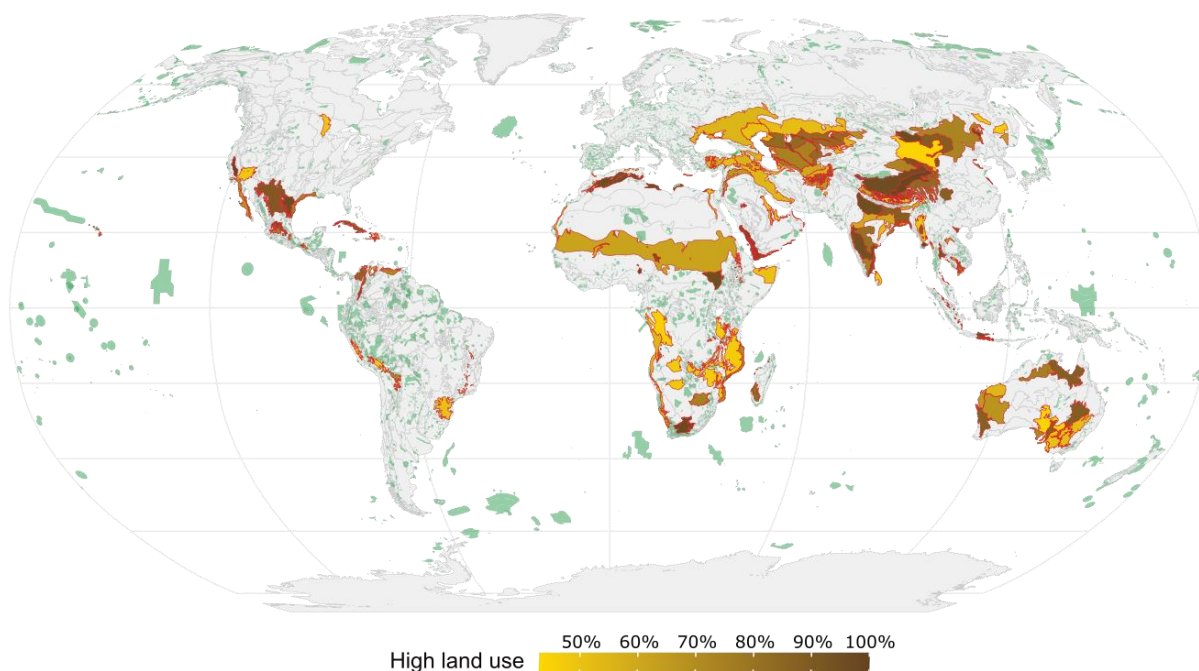
413 flooded grasslands, tropical and subtropical coniferous forests, and tundra, were
 414 consistently underrepresented in all methods. GLAM3 was based on the most extensive
 415 set of studies regarding biomes, integrating studies conducted in all of them (Table S3).
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 418 *Figure 4. Representativeness of terrestrial biomes in the studies underlying the four biodiversity*
 419 *impact assessment methods (IBIF/GLOBIO, GLAM3, IW+, and ReCiPe 2016), compared with the*
 420 *global biome distribution by area (Olson et al. 2001). Bars show the relative proportion of studies*
 421 *assigned to each biome, based on unique study–biome combinations. Percentages are shown only*
 422 *for the most dominant biome categories per method (See Table S2 for percentages for all biomes).*
 423

424 To identify priority regions for future research, we overlaid study coordinates from the four
 425 methods with data on key biodiversity areas (KBAs) and anthropogenic land use. We
 426 found that methods included studies for 274 ecoregions containing both KBAs and high
 427 levels of anthropogenic land use, while 358 ecoregions were not represented by any study
 428 included in the four methods. Coverage varied considerably among methods: GLAM3
 429 exhibited the highest coverage, representing 40% of the ecoregions, whereas IBIF had the
 430 lowest coverage at only 11% (Fig. S3).
 431

432 Among the 358 ecoregions not represented by any study in the underlying evidence base
 433 of the four assessed methods, we identified 150 priority ecoregions for future research,
 434 corresponding to approximately 40% of all unrepresented ecoregions, to provide a concise
 435 and globally representative set of research priorities. These were selected based on the
 436 presence of Key Biodiversity Areas (KBAs) and exhibiting the highest levels of
 437 anthropogenic land-use pressure, measured as the proportion of anthropogenically used
 438 land within each ecoregion (Fig. 5). Priority regions were concentrated primarily in sub-
 439 Saharan Africa, Central America, South and Central Asia and Australia.
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4. Discussion

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Figure 5. 150 priority ecoregions for future research. Priority ecoregions were identified as those lacking studies across all four methods considered, while also containing Key Biodiversity Areas (KBAs) and exhibiting high anthropogenic land use analysed as the number of high land use pixels ($>50 \text{ km}^2$ per grid cell) (cropland, pasture, grazing land, urban areas) relative to total number of pixels in the ecoregion. Red boundaries delineate priority ecoregions, green areas show KBAs outside priority ecoregions, and grey areas represent all remaining ecoregions.

The CFs derived from the methods assessed in this study are widely used in LCA and other applications. Consequently, limitations in the underlying knowledge base are propagated across all biodiversity assessment tools that rely on them. We found that overall, the methods assessed differed in their evidence bases. Pronounced taxonomic and geographic biases were found in all methods with most studies covering well-studied groups such as vascular plants, mammals and birds.

4.1 Empirical evidence underpinning the biodiversity footprint assessment methods

We found that the information of publications underpinning the CFs was not always reported transparently. For instance, both ReCiPe and IW+ rely on meta-analyses, but these publications did not provide complete reference lists. For this study, we obtained the references through requesting them directly from the authors of the meta-analyses (de Baan et al., 2013; Elshout et al., 2014). Greater transparency regarding the underlying studies is essential for evaluating the reliability and reproducibility of the methods and the CFs they generate.

470 In this study, we focused only on pressure-impact relationships for land use, which is one
471 of the most studied anthropogenic drivers of biodiversity loss. The potential biases
472 stemming from the selection of included studies for the other, often less studied pressures
473 such as water use, invasive species and pollution, remain an equally important aspect to
474 be assessed and developed and we encourage future studies to investigate them.

475
476 The development of CFs in some of the methods was informed by publications conducted
477 decades ago. For example, ReCiPe and IW+ both also included empirical studies dating
478 back to the 1970s. The estimated pressure-impact relationship does not necessarily
479 become outdated, but human pressures have intensified rapidly during the past decades
480 (IPBES, 2026), and a vast number of new empirical studies, meta-analyses and
481 biodiversity monitoring data has since become available (Steinke et al., 2025; Heberling et
482 al., 2021; Keck et al., 2025). For instance, when comparing the publications included in the
483 four methods and the extensive meta-analysis on human impacts on biodiversity by Keck
484 et al. (2025), we found that only a fraction of the publications included in Keck et al. had
485 been included in building the CFs of the four methods. Although we did not systematically
486 evaluate the compatibility of these studies for estimating CFs, this gap suggests that a
487 substantial share of available empirical evidence remains unincorporated into current
488 characterisation methods, including many studies addressing often less well-represented
489 taxonomic groups such as microbes and fungi. This highlights both the need and the
490 opportunity for systematic updates of existing CFs methods by routinely expanding the
491 underlying study basis.

492
493 The robustness of the CFs of these four methods is further compounded by differences in
494 how uncertainty is quantified and reported. IBIF and GLAM3 do not provide uncertainty
495 ranges for the CFs (Scherer et al., 2023; Schipper et al., 2025), but in GLAM3 the spatial
496 uncertainties due to aggregation from ecoregions to countries or global level, i.e. the
497 relative weighted standard deviations, are reported. ReCiPe and IW+ present the CFs with
498 uncertainty ranges, but they use different approaches for deriving these ranges and thus
499 make it difficult to compare pressure-impact assessments conducted with those methods.
500 In IW+, the range depicts uncertainty due to spatial variability when disaggregating from
501 biome level to country level (Bulle et al., 2019). In ReCiPe, on the other hand, the different
502 sources of uncertainty and different methodological choices were grouped into three
503 scenarios, consequently providing three sets of endpoint CFs and encouraging the users
504 to use all of them for sensitivity testing of their LCA results (Huijbregts et al., 2016).

505 506 4.2. Biases in the knowledge base

507
508 Our findings show that there are clear taxonomic and geographic biases in the knowledge
509 base used to define CFs for the four methods. This limited representativeness of the
510 empirical data can lead to increased uncertainty of pressure-impact estimates relying on
511 them. This is particularly problematic when relationships are extrapolated to taxa or
512 regions that are underrepresented in the original studies (Bromwich et al., 2025).

513
514 Different taxonomic groups respond differently to land use due to variation in their
515 ecological requirements for access to resources, habitat conditions, and opportunities for

516 reproduction. They also vary substantially in their life-history traits and dispersal
517 capacities. As a result, models calibrated primarily on well-studied taxa may fail to capture
518 the responses of less-studied groups, such as many invertebrates and fungi. At the same
519 time, inconsistencies can also arise from the opposite imbalance. For example, in the
520 GLAM3 method, 54% of the studies informing pressure–impact relationships for animals
521 focus on invertebrates, yet the resulting CFs for animal groups are developed only for
522 vertebrates including amphibians, birds, mammals, and reptiles. This mismatch may
523 introduce additional uncertainty, as invertebrates can respond differently to land-use
524 changes compared to vertebrates.

525
526 Similarly, geographic biases limit the ability of models to accurately represent biodiversity
527 responses when applied for under-sampled regions and may lead to systematic over- or
528 underestimation of biodiversity impacts in these regions. Consequently, the CFs derived
529 from a biased set of studies may not be fully transferable across contexts, reducing their
530 reliability in assessing the impacts across complex global supply chains. As a result,
531 biodiversity impacts may be estimated using characterisation factors that do not
532 adequately reflect local ecological conditions, potentially biasing comparisons between
533 products, supply chains, or sourcing strategies.

534
535 Furthermore, the common practice in all four methods, to aggregate taxonomic groups,
536 may result in challenges to defining robust CFs. For example, when estimating land use
537 impacts on plants, de Baan et al. (2013) showed that separating them into subgroups of
538 mosses and vascular plants resulted in differences in the biodiversity impact estimates and
539 consequently different values for CFs for these subgroups. Yet, in all four assessed
540 methods, CFs for plants were modelled as one group by combining studies of moss and
541 vascular plants (Table S1).

542 543 4.3. Pathways for improvement

544
545 The limited representativeness and biases that we have uncovered in this study regarding
546 the knowledge base of the commonly used biodiversity impact assessment methods,
547 ultimately affects biodiversity footprint assessments that rely on them. They have the
548 potential to skew results on biodiversity footprinting and misinform decision-making. For
549 transparency, robustness, and comparability, it is crucial to clarify which taxonomic groups,
550 biomes, or geographical dimensions the reported impacts actually represent, as well as to
551 make explicit the associated uncertainties and underlying knowledge gaps. Addressing the
552 limitations requires improved transparency by method developers and users, increased
553 incorporation of underrepresented taxa and regions by method developers, focused
554 studies by researchers studying biodiversity impacts, and the development of methods to
555 explicitly account for uncertainty and variability in ecological responses (Table 2).

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Table 2. Recommendations for improving the robustness and representativeness of biodiversity impact characterisation factors.

Theme	Recommendation	Main responsible actors	Supporting actors	Expected outcome
Responsible interpretation and use	Clearly communicate what biodiversity dimensions CFs represent, their limitations, assumptions, and appropriate applications. Encourage sensitivity analyses and critical assessment of method suitability for the decision context.	Method developers	LCA practitioners, researchers	More informed use of CFs and reduced risk of drawing misleading conclusions from quantitative biodiversity footprint results.
Expansion of the empirical knowledge base	Prioritise data collection in underrepresented taxa, biomes, regions, and land-use contexts, and mobilise underused data sources such as environmental impact assessments conducted by companies and public institutions.	Researchers, research funders, companies, public authorities	Database developers, conservation organisations	A more representative evidence base and reduced uncertainty due to smaller data gaps and biases.
Standardisation of biodiversity data collection and reporting	Establish common reporting standards and metadata requirements for empirical studies, including biodiversity observations, sampling methods, location, habitat characteristics, and land-use intensity, while ensuring that reporting meets the needs of different biodiversity indicators.	Researchers, database developers, standard-setting organisations	Journal editors, publishers, funders, method developers	Improved interoperability and easier integration of new data into biodiversity impact assessment models.
Continuous updating and responsible use	Regularly update databases, models and CFs as new evidence becomes available, and encourage users to assess sensitivity across methods while reporting uncertainties and limitations alongside results.	Method developers, database developers, LCA practitioners	Researchers, decisionmakers, companies	Methods that remain scientifically robust and support informed action despite unavoidable uncertainty.

561

562 Uncertainty will always remain because every model inevitably involves choices and value-
563 based assumptions. It is therefore essential to examine these uncertainties carefully to
564 avoid drawing suboptimal conclusions. Concerning biodiversity impact assessments, one
565 approach is to provide an uncertainty range for the CFs, to reflect different methodological
566 and value choices, and to report impact assessment results as ranges rather than single
567 values. Explicit treatment of uncertainty would also enable decision analyses in which
568 quantifying the risk of an unacceptable outcome (such as ecosystem collapse) can be
569 more critical than identifying the single most likely outcome (Burgman 2005). Combining
570 results from multiple methods to test their sensitivity has also been recommended
571 (Bromwich et al., 2025; Martínez-Ramón et al., 2025). Yet, as many methods rely on the
572 same underlying knowledge base, this might constitute pseudoreplication, which may
573 partly limit the benefits of their combination. For example, the study of de Baan et al.
574 (2013) has been used to develop the CFs in both IW+ and ReCiPe and is further based on
575 data from an earlier version of the GLOBIO model (Alkemade et al., 2009). Similarly, both
576 IBIF and GLAM3 rely on PREDICTS database (Hudson et al., 2017) as their empirical
577 foundation (Fig 1).

578
579 Beyond improving the characterisation of uncertainty, there is also a need for greater
580 clarity regarding how CFs should be interpreted and applied in practice. Although
581 biodiversity CFs are increasingly used in LCA studies and decision-support applications,
582 the methodological assumptions, taxonomic coverage, spatial representativeness, and
583 underlying biodiversity metrics that the impacts are quantified (e.g. PDF, MSA) often
584 remain poorly understood by end users. As a result, CFs may be treated as universally
585 applicable parameters despite being derived from specific datasets and ecological
586 indicators and being based on different value choices. This can create a false sense of
587 precision, whereby quantitative results appear robust and directly comparable even when
588 they are based on fundamentally different conceptualisations of biodiversity impacts. Clear
589 guidance from method developers, along with transparent reporting of the scope,
590 limitations, and intended use of CFs, is therefore essential. Users should be encouraged to
591 interpret biodiversity footprint results as decision-support information rather than precise
592 estimates of real-world biodiversity loss, and to critically assess whether the chosen
593 method is appropriate for the decision context and biodiversity dimensions of interest.

594
595 One fundamental issue in the application of both carbon and biodiversity footprints of land
596 use is the incoherent choice of reference land use, i.e. the baseline against which land use
597 impacts are quantified (Soimakallio et al. 2015, 2025). In all methods considered in this
598 paper, reference areas in the empirical data underlying the CFs essentially represent
599 current (semi)natural habitats, although the verbal definition of the reference land use
600 varies between methods (Table 1). According to Soimakallio et al. (2025), from this follows
601 that the methods are implicitly designed to coherently assess the effects of land use
602 compared to leaving the area in its natural state. However, for areas already in intensive
603 human use, a more relevant question to inform future management decisions may often be
604 how the continued use of land will further affect biodiversity, compared to discontinuing its
605 use. To allow such decision-supporting analyses, it would be necessary to extend the
606 underlying data to also include reference sites representing natural regeneration from a
607 modified state back to a more natural state.

608

609 Integrating data from additional studies to address the knowledge gaps identified in this
610 paper would strengthen method robustness and help reduce uncertainties arising from
611 limited or biased data coverage. Potentially underutilised sources of data include the
612 environmental impact assessments conducted by companies and public institutions, e.g.,
613 municipalities and cities (GBIF Secretariat & IAIA, 2020). However, these data are often
614 scattered across different organisations and not readily accessible for research purposes.
615 Indeed, the gap between production of biodiversity-impact data and its usefulness for
616 decision-making and impact assessment has been recognised earlier and a Biodiversity
617 Monitoring Standards Framework has been suggested at the broader level to guide
618 biodiversity data collection fit for purpose (Gonzalez et al., 2026).

619

620 To improve the potential of incorporating more data and fill the gaps identified here, new
621 empirical studies, assessments, and meta-analyses collating data from field studies should
622 report the necessary variables for pressure-impact estimation in a transparent and
623 standardised manner. Different indicators require different types of information from
624 empirical studies. GLAM3, ReCiPe and IW+ quantify relative species richness, for which
625 the key requirement is reporting the number of species observed at each sampling site,
626 together with as detailed a taxonomic description as possible of the sampled species
627 groups. IBIF, in contrast, measures mean species abundance, and therefore requires a
628 complete species list, identification of native or original species, and their relative
629 abundances. In addition, all studies should carefully document essential metadata such as
630 sampling time, sampling methods, geographic coordinates, habitat characteristics, and
631 land-use intensity. In addition, for reference areas representing natural regeneration, the
632 time since the discontinuation of land use is required.

633

634 Finally, it is important to remember that uncertainties in biodiversity impact assessments
635 do not arise solely from the characterisation factors. Even with robust characterisation
636 models, assessment results remain constrained by the quality of the underlying inventory
637 data, as well as how well inventory flows can be matched to the characterisation factors
638 (Scherer et al., 2021; Bromwich et al., 2025). While uncertainty can never be fully
639 eliminated, it should not be used as a justification for inaction (Martínez-Ramón et al.,
640 2025; IPBES, 2026). We emphasise that despite their limitations, current methods already
641 offer valuable guidance for actors seeking to understand and reduce their impact. As new
642 information accumulates on both human pressures and their impacts on biodiversity, the
643 evidence base for biodiversity impact characterisation should be continually updated and
644 strengthened. Doing so would help ensure that methods remain robust and that
645 uncertainties linked to incomplete or biased data are progressively reduced.

646

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Author contributions

VS, MH, and VN conceived and designed the study. VS developed the methodology, conducted the analyses, and prepared the visualizations with the help of the co-authors. All authors contributed to methodological discussions, interpretation of the results, and refinement of the research and analysis. VS led the writing of the manuscript, with input and revisions from all authors.

Data availability statement

The publications underpinning the four methods assessed in this study are listed in Supplementary Data.

The original data underlying land use characterization factors in GLAM3 are publicly available through Zenodo (<https://doi.org/10.5281/zenodo.10114493>). For IBIF, the publications used to derive the land use pressure–impact relationships are documented in Annex S1 of Schipper et al. (2020), and the associated data are available through the PREDICTS database (Hudson et al., 2023; <https://doi.org/10.5519/j4sh7e0w>). The datasets underlying ReCiPe and IW+ were obtained directly from the authors of De Baan et al. (2013) and Elshout et al. (2014), respectively. The dataset associated with Keck et al. is publicly available through Zenodo (<https://doi.org/10.5281/zenodo.14608770>).

Conflict of interest

Laura Scherer contributed to the development of the GLAM3 land-use characterisation factors evaluated in this study. The authors declare no other competing interests.

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