Enigmatic persistence of dissolved organic matter in the ocean

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Abstract | Marine dissolved organic matter (DOM) contains more carbon than the combined stocks of Earth's biota. Organisms in the ocean continuously release a myriad of molecules that become food for microheterotrophs, but, for unknown reasons, a residual fraction persists as DOM for millennia. In this Perspective, we discuss and compare two concepts that could explain this persistence. The long-standing 'intrinsic recalcitrance' paradigm attributes DOM stability to inherent molecular properties. In the 'emergent recalcitrance' concept, DOM is continuously transformed by marine microheterotrophs, with recalcitrance emerging on an ecosystems level. Both concepts are consistent with observations in the modern ocean, but they imply very different responses of the DOM pool to climate-related changes. To better understand DOM persistence, we propose a new overarching research strategy — the ecology of molecules — that integrates the concepts of intrinsic and emergent recalcitrance with the ecological and environmental context.

The average litre of seawater contains less than 1 mg of dissolved organic matter (DOM), but these DOM traces add up to ~660 petagrams of carbon — more carbon than in Earth's marine and terrestrial biota combined[1]. The global DOM pool contains not only vast quantities of carbon but also nitrogen, phosphorus, iron and other elements essential to life[2]. As such, DOM is a key component of marine biogeochemical cycles[2]. However, the role of the DOM pool in the climate system remains speculative[3]. In contrast to forests and soil carbon (for example)[4], active management of the DOM pool to mitigate climate change remains far out of reach. Indeed, the majority of observable DOM in the ocean has an estimated lifetime of 16,000 years[5], and, therefore, was formed long before industrialization. With atmospheric carbon dioxide concentrations and global temperature rising further in the next few decades, the ocean will become warmer, less basic, less oxygenated and more stratified[6]. There is a fundamental lack of knowledge on whether and how the DOM pool will respond to these changes[7].

Microorganisms are the main engine for marine biogeochemical cycles[8,9]. Marine phytoplankton is responsible for about half of the global net primary production[2]. In contrast to terrestrial environments, little carbon is stored in living biomass in the ocean[2] and marine organisms release a myriad of organic compounds while they live and upon death. Most DOM released by phytoplankton is quickly consumed by microheterotrophs (heterotrophic bacteria, archaea and eukaryotic microorganisms), and is turned over within hours to days[10,11]. The microbial loop[8,9] — the continuous cycling of fixed carbon through DOM release and microbial turnover (FIG. 1) — converts up to 40 petagrams of carbon each year[2]. A minor fraction of the released DOM apparently escapes immediate utilization, decomposing slowly enough to be transported by currents across the globe for thousands of years, surviving several global ocean meridional overturns[1].

The environmental context is paramount for the turnover of organic matter[4,12,13]. Major deposits of organic matter on Earth have accumulated under conditions

unfavourable for life, such as excessive temperature in deep sediments[12] or lack of liquid water in permafrost deposits[14]. Also, the lack of electron acceptors (such as oxygen or sulfate) can make heterotrophic life thermodynamically unfeasible[12]. In the oceanic water column, environmental conditions vary on all scales (FIG. 2), from micrometres to kilometres and within minutes to seasons. For instance, sinking particles can be suboxic in their centre while the surrounding water column is oxic[15], exposure to solar radiation in the sea surface microlayer drives photochemical reactions[16] and iron concentrations vary over more than one order of magnitude between ocean regions[17]. Spatial heterogeneity of essential elements, like enzymatic cofactors[18], can slow DOM decomposition over months to decades, and the highest surface concentrations of DOM occur mainly in nutrient-poor stratified waters, such as in the cores of subtropical gyres or in late summer in temperate regions[19,20]. There are also large-scale biogeographical patterns in the substrate affinity of marine bacteria. Bacterioplankton take up certain groups of saccharides in one region of the surface ocean but prefer others elsewhere[21]. It has been proposed that, because of biogeographical patterns in substrate affinity, DOM considered recalcitrant in one part of the ocean is degradable elsewhere[22,23].

However, environmental conditions are likely not the sole reason for long-term DOM persistence[7]; in the deep ocean, heterotrophic life is limited by the availability of organic carbon and, as far as is known, not by other missing components[24,25]. Microorganisms could obtain energy[26] and nutrients via the oxidation of DOM, because these chemically reduced compounds are in thermodynamic disequilibrium in the oxygen-replete and nutrient-replete deep ocean. Paradoxically, microheterotrophs that depend on the supply of organic matter for their survival are surrounded by an enormous pool of DOM that appears to remain untouched for thousands of years.

Several hypotheses explain the paradox of DOM persistence in the ocean[7]. The long-standing 'intrinsic recalcitrance' paradigm states that lability and recalcitrance are intrinsic, molecular
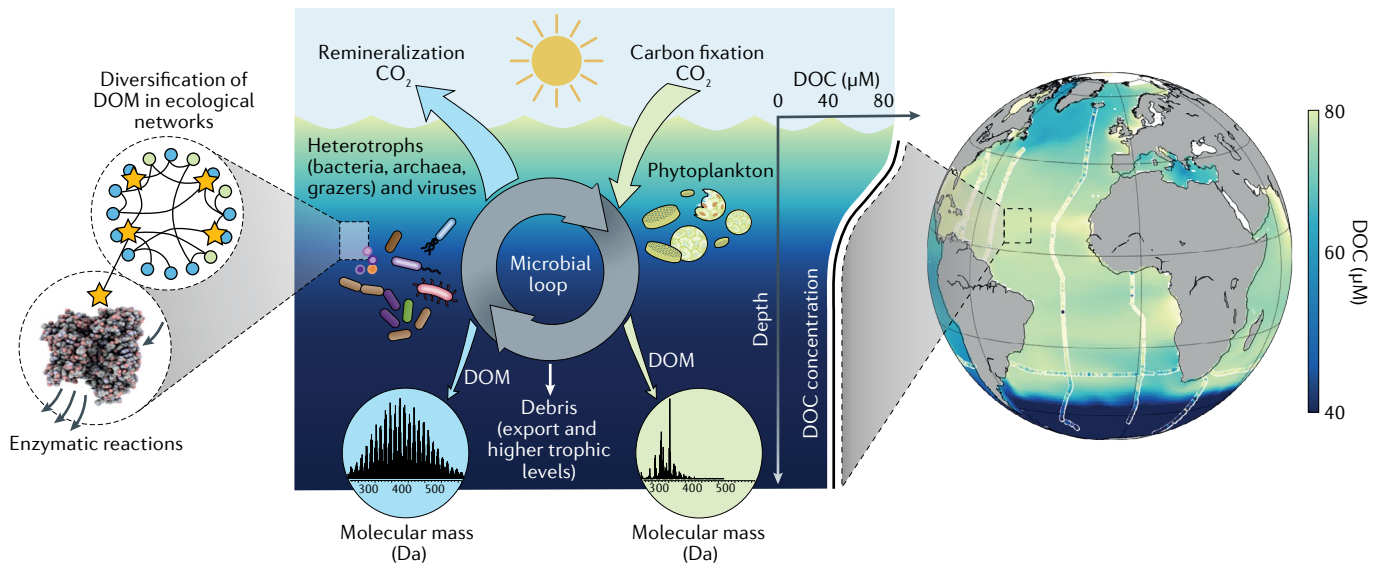
Fig. 1 | **Small-scale processes drive global patterns of dissolved organic matter.** After formation by phytoplankton, most dissolved organic matter (DOM) is consumed by microheterotrophs, starting the microbial loop (central panel)[10,11]. Up to 40 petagrams of carbon is turned over every year in this microbial loop[2,8,9]. A fraction of DOM apparently escapes the microbial loop, decomposing slowly enough to accumulate and move with currents across the globe for thousands of years[1]. At the smallest scale (left panel), DOM is molecularly diversified during microbial degradation through a myriad of enzymatic reactions (yellow stars), forming a complex degradation network where algae-derived DOM compounds (green circles) are continuously transformed into other labile and recalcitrant DOM compounds (blue circles). The ultrahigh-resolution mass spectra (molecular mass versus signal intensity) shown in the circular inserts illustrate the high level of molecular diversity of deep-sea DOM (blue insert) compared with algae-derived DOM (green insert). Each line in the spectra represents a distinct set of dissolved organic molecules. About 20,000 molecular formulae have been identified in deep-sea DOM through this approach[68]. At the scale of the global ocean (right panel), DOM is not evenly distributed. The colour code of the global sketch corresponds to approximate sea surface concentrations of dissolved organic carbon (DOC)[19], whereas the depth profile is typical for most parts of the stratified ocean[2]. The lines on the globe are cruise tracks where observed DOC concentrations are publicly available[199].

properties of DOM[27–30]. There is evidence that microorganisms synthesize biologically recalcitrant molecules while they consume labile compounds[31], constituting a microbial carbon pump through which carbon is sequestered from active cycles[32]. Photochemical reactions at the sea surface and other abiotic processes could also contribute to the formation of intrinsically recalcitrant compounds[33,34].

A newer, alternative concept proposes the continuous transformation of all forms of DOM. According to this concept, recalcitrance is not an intrinsic property of DOM but emerges on an ecosystems level from ecological interactions[35,36]. By analogy, it was recently proposed that the persistence of soil organic carbon is caused by functional complexity and likely emerges from nonlinear interactions among decomposers, diverse organic substrates, and the heterogeneous and dynamic soil environment[4]. Properties that individual parts of a complex system do not have on their own emerge when they interact in a larger whole[37]. According to this 'emergent recalcitrance' concept, DOM constituents and microorganisms are individual players within ecological networks. Within the resulting microbial degradation network,

microbial growth and DOM degradation rates slow as concentrations decline.

In this Perspective, we combine insights from geochemistry, biochemistry, microbiology, ecology and mathematical modelling. We apply this knowledge to assess DOM persistence, discussing the two central concepts of intrinsic recalcitrance and emergent recalcitrance. The actual mechanism for DOM persistence could be a combination of both concepts, and additional mechanisms are likely at work. At the end of this Perspective, we propose the 'ecology of molecules' as a new overarching research strategy to solve the enigma of DOM persistence. This unifying strategy integrates the concepts of intrinsic and emergent recalcitrance within the ecological and environmental context.

DOM patterns and properties

Phytoplankton are the primary source of DOM in the ocean[1,38,39]. Indeed, in mesocosm experiments, a small fraction (<0.4%) of planktonic net community production is channelled by marine microbial communities into a form of DOM that is molecularly indistinguishable from refractory DOM in the deep sea[40]. A diversion of 0.4% appears as a minor,

almost insignificant, fraction of marine production, but it is sufficient to balance the annual loss of refractory DOM in the global ocean[40,41]. As the primary source of DOM is phytoplankton, DOM attains the highest concentrations (60–90 μM carbon) in the productive surface layer (FIG. 1). Coastal upwelling systems, where nutrient-rich waters of the deep ocean reach the sunlit surface ocean, are hotspots of DOM production[42]. DOM concentrations diminish with water depth, with the lowest values (34–48 μM carbon) in the deep waters (>1,000 m) (FIG. 1). Global oceanic circulation shapes the DOM distribution[43,44]. Deep water formation at high latitudes, and winter convective mixing, export surface water enriched in DOM to greater depths[5]. In the deep ocean, concentrations decline by >10 μM carbon (~20%) along the main pathways of deep ocean currents from the North Atlantic to the North Pacific Ocean, coincident with the millennial age gradient of the deep ocean water mass[45].

Global ocean models[1,39] and the stable carbon isotopic composition of DOM[38] indicate that non-planktonic sources are only quantitatively important at the continental margins and potentially at the seafloor. For example, hydrothermal systems

are potential formation sites of DOM, but this source is minor compared with most oceanic DOM sources[46,47]. A fraction of organic matter produced by biota on land enters the ocean via rivers and groundwater after its journey across landscapes and through soils. This flux is sufficient to sustain the turnover of the entire DOM pool in the ocean[48]. However, decomposition products from land plants, such as lignin-derived phenols, are scarce in marine DOM; <2.5% of the total DOM in the ocean appears to be terrigenous[49]. Globally, about 10% of riverine DOM are polycyclic aromatics stemming from charcoal buried in soils[50]. Even this presumably stable compound group is either mineralized or removed from solution following discharge to the ocean[51].

Age and turnover. From DOM concentration gradients and water mass ages, turnover rates of operationally defined DOM fractions can be deduced[5,52] (FIG. 3). Recalcitrant DOM, in contrast to labile DOM, is the fraction that accumulates in the ocean and, thus, is analytically observable[5]. Recalcitrant DOM tends to group into various fractions of apparent reactivity: semi-labile DOM (removal within weeks to months, mainly in the upper ocean), semi-refractory DOM (decadal removal) and refractory DOM (millennial removal). Refractory DOM, at a global ocean inventory of ~630 petagrams of carbon, is the fraction that escapes observable microbial removal[5]. Its renewal rate in the subsurface ocean is estimated at 0.043 petagrams of carbon per year; the corresponding lifetime is 16,000 years[5]. DOM in the ocean is a mix of all fractions, none of which is currently analytically separable. The concept of recalcitrant reactivity classes does not infer specific mechanisms behind the observed turnover.

Radiocarbon dating supports the concept of a very old and ageing pool of DOM in the deep ocean, reaching apparent radiocarbon ages of up to 6,000 years[53–55]. This age is 'apparent' because DOM is a mixture of fractions of various ages and turnover times[56] (FIG. 3). In the upper mixed layer of the ocean, DOM appears much younger (1,000–4,000 years)[57], owing to the additions of modern carbon. Concentration and radiocarbon profiles can be reproduced in a simple two-component model that includes a variable amount of young, semi-labile DOM cycling in the upper ocean (<1,000 m), superimposed on a ubiquitous background of old refractory DOM[58–60]. One model suggests that DOM in the deep ocean is not uniform but isotopically diverse, with a

broad range of potential sources and cycling timescales[56]. Some components of the deep DOM reservoir are modern, whereas others have radiocarbon ages exceeding 10,000 years[56].

Importantly, age and turnover rates do not necessarily correspond. For instance, DOM in the deep Mediterranean Sea is older than in the deep Atlantic Ocean, but a substantial fraction of traditionally defined 'refractory' DOM imported from the Atlantic Ocean is removed in less than 100 years[61]. At the same time, pre-aged DOM from sedimentary or terrestrial sources might be added to the Mediterranean Sea DOM pool[61]. Thus, even the 'refractory'

component of DOM, with an apparently long lifetime in the deep ocean, comprises a diverse and more actively cycled pool[56,61] than previously assumed[5,58].

Molecular properties. Freshly produced, phytoplankton-derived DOM is largely composed of biomolecules of known building blocks, most prominently polysaccharides, proteins, lipids and DNA[2,62,63]. Notably, algae produce >10 petagrams per year of the polysaccharide laminarin, which has a comparably simple structure and is turned over on a diurnal timescale[63,64]. By contrast, a major fraction of deep-sea DOM has a universal structural
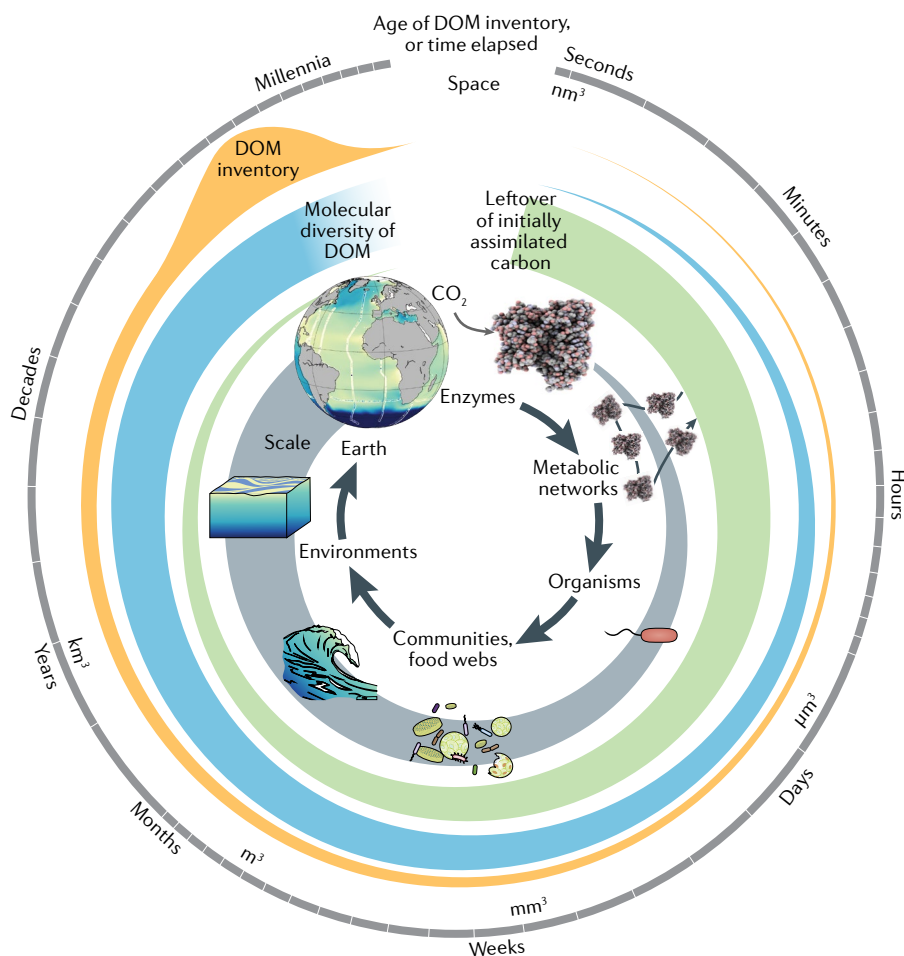

Fig. 2 | **Formation and degradation of dissolved organic matter across spatial and temporal scales.** Formation and degradation of dissolved organic matter (DOM) is the result of enzymatic reactions that scale up to global effects, bridging more than ten orders of magnitude in space and time. The clockwise circular scale denotes space scales, time elapsed after photosynthetic carbon fixation and age of the DOM inventory. As organisms release DOM during growth and upon death[116], about half of the net primary production is quickly turned over by microorganisms within hours to days[10,11,116] (green band). This freshly produced, phytoplankton-derived DOM is largely composed of biomolecules of known building blocks, such as polysaccharides[62,63]. During microbial decomposition, DOM is molecularly diversified[106] (blue band). All relevant facets of molecular diversity cannot yet be analysed, especially that of the most persistent fraction of DOM[67]. A minor fraction of the released DOM escapes the microbial loop, decomposing slowly enough to persist in the ocean for millennia, making most of the DOM pool old[2] (orange band).

a DOM inventory

l-DOM:
0.2 Pg

sl-DOM:
6 Pg

sr-DOM
14 Pg

ur-DOM:
12 Pg

r-DOM
630 Pg

Age of DOM inventory or time elapsed

Seconds   Hours   Weeks   Years   Decades   Millennia

b Leftover of initially assimilated carbon

Emergent recalcitrance concept

Continuous microbial
reworking on all scales

Intrinsic recalcitrance concept

Formation of
refractory DOM

Abiotic removal
of refractory DOM

Transient
DOM

Labile
DOM

Refractory
DOM

c DOM degradation: rate limiting factors

Emergent recalcitrance concept

Organisms
Ecological interactions
Traits

DOM
Concentration of constituents
Molecular structure

Intrinsic recalcitrance concept

Organisms
Ecological interactions
Traits

DOM
Concentration of constituents
Molecular structure

Fig. 3 | **Two central concepts of dissolved organic matter persistence in the ocean.** The actual mechanism could be a combination of both, and additional mechanisms are likely at work. The time and age of dissolved organic matter (DOM) advances on a logarithmical scale, from seconds to millennia, from left to right here. **a** | The majority of observable DOM in the ocean has an estimated lifetime of 16,000 years and is refractory by definition[5]. **b** | Fate of initially assimilated carbon. In the emergent recalcitrance concept, microheterotrophs continuously transform all forms of DOM. Recalcitrance emerges on an ecosystems level from ecological interactions and degradation (indicated by the size of the circular arrows) slows as molecular diversity increases and concentrations decline[35,36]. The intrinsic recalcitrance paradigm states that lability and recalcitrance are intrinsic, molecular properties of DOM[27–30]. Presumably, microorganisms synthesize biologically recalcitrant molecules while they consume labile ones[31], constituting a microbial carbon pump through which carbon is sequestered from active cycles[32]. Refractory DOM is returned to active cycles largely via abiotic processes, such as photochemical reactions at the sea surface[76,100], although abiotic removal can occur in both concepts. Transient DOM refers to the semi-labile and semi-refractory fractions together. **c** | Rate-limiting factors for DOM degradation on the different timescales. On timescales of phytoplankton and bacterioplankton blooms, ecological interactions, traits of organisms, substrate composition and substrate concentration are all crucial for DOM turnover. According to the emergent recalcitrance concept, long-term turnover is largely controlled by properties of organisms and ecosystems; the molecular structure of DOM is a secondary control. According to the intrinsic recalcitrance paradigm, the long-term fate of DOM is largely determined by molecular properties of DOM. The molecular structure shown is an example of a proposed recalcitrant structure in the ocean[85]. l, labile; r, refractory; sl, semi-labile; sr, semi-refractory; ur, ultra-refractory.

Intrinsic recalcitrance

The reasons behind the persistence of DOM are unknown. It is a long-standing paradigm that lability and recalcitrance are intrinsic, structural properties of DOM[28,71,72]. Therefore, in the intrinsic recalcitrance concept, long-term persistence can be explained if some compounds are only very slowly or not at all consumed by microorganisms[32]. Microheterotrophs play a major role in this concept by potentially converting simple biomolecules from primary producers into biologically refractory DOM[31]. Some scenarios involve abiotic processes, in particular light-induced and heat-induced reactions that could produce molecular structures resistant to microbial degradation[33,73,74]. Removal of biologically refractory DOM is largely driven

composition across the globe[65], and most dissolved organic compounds have low molecular mass (<1,000 Da)[66].

DOM is among the most complex mixtures on Earth[67]. The level of chemical diversity challenges even the most advanced analytical techniques, such that the molecular structure and concentration of only a minor fraction of all the compounds present in DOM is known[2]. Exact estimates on the number of different compounds in DOM are lacking, but more than

20,000 molecular formulae have been identified with ultrahigh-resolution mass spectrometry[68] (FIG. 1), with 30 or more isomers per formula[67]. Combining these estimates sum to >600,000 different compounds, but there are possibly many millions of different organic compounds in DOM[69], each presumably below picomolar concentrations. For comparison, the total number of possible small organic molecules in the universe has been estimated to exceed 10^{60} (REF.[70]).

by abiotic processes such as adsorption onto sinking particles[38], photodegradation[75–77] or entrainment in the hydrothermal circulation within Earth's crust[46] (FIG. 3). To assess the role of DOM in the Earth system, the intrinsic recalcitrance concept is usually implemented into biogeochemical ocean circulation models by assuming certain conversion efficiency from labile to refractory DOM (FIG. 4); some models also consider a form of transient, semi-refractory DOM[78–80]. The modelled DOM reactivity pools decay at prescribed, fixed rates, following first-order kinetics like radioactive decay[79,81–83] (BOX 1).

Lines of evidence. There are three major lines of evidence in support of the 'intrinsic recalcitrance' concept. First, systematic changes in DOM composition with water mass age suggest a linkage between the stability and molecular structure of DOM compounds, because some compounds accumulate over time more than others[28,84,85]. For instance, refractory DOM contains structural polymers that are more resistant to hydrolysis and enzymatic attack than other common biopolymers[28], especially cell-wall-specific D-amino acids[30,86], amino sugars[87] or fatty acids[88]. DOM also contains non-hydrolysable, acylated polysaccharides[89,90] that persist after more labile DOM has been degraded. However, most of DOM is molecularly uncharacterized and the extrapolation of information from molecular tracers to the bulk of DOM introduces inherent uncertainties.

Second, DOM represents a heterogeneous mixture of compounds of different ages, indicating that some compounds are cycled faster than others, even in the deep sea[56,60,84]. The radiocarbon ages of the high-molecular-mass fraction and hydrolysable carbohydrates of DOM in the ocean are much younger than that of bulk DOM[91,92], consistent with their reactivity in microbial incubation studies[93]. Moreover, small and chemically resistant compounds are among the oldest in DOM[57,60], including lipophilic compounds[60] and possibly degraded microbial lipids[94], with some turning over on an apparent timescale of up to 30,000 years[56]. Input of pre-aged DOM, possibly from ancient deposits or hydrothermal circulation, introduces some uncertainty[94,95], but there is consensus about a linkage between apparent radiocarbon age and molecular structure in marine DOM.

Third, microheterotrophs prefer certain substrates. This substrate affinity exhibits a major control on organic matter turnover

in bloom situations[96], and it was suggested that organic matter turnover at millennial timescales is also linked to substrate preferences of microheterotrophs[97]. Abiotic transformations, such as photochemical reactions at the sea surface, influence substrate quality. After light exposure, some previously labile compounds resist microbial degradation over months, while some refractory compounds become partially bioavailable[33,34,77,98,99]. The light-induced formation of recalcitrant structures might be due to crosslinking of polyunsaturated compounds or radical-mediated photo-oxidation of any functionalized molecule[74,85]. At the same time, bioavailable, low-molecular-mass compounds are likely cleaved off refractory DOM[76]. This proposed removal mechanism could counterbalance the microbial formation of refractory DOM[76,100].

Overall, there is broad consensus that, on the timescales of days to years, substrate composition, substrate availability, microbial traits and ecological interactions govern DOM turnover (FIG. 3) and control the succession of microheterotrophs in bloom situations in the ocean[96]. The intrinsic recalcitrance concept goes a step further, postulating intrinsic stability of some microbially produced compounds or abiotically transformed derivatives on much longer timescales[71].

Limitations. Microorganisms evolved mechanisms to degrade even the most complicated molecules on Earth, and we are unaware of biomolecules that resist microbial degradation under favourable environmental conditions. The occurrence of enzymes dedicated to degradation of plastics suggests that even new enzymes can
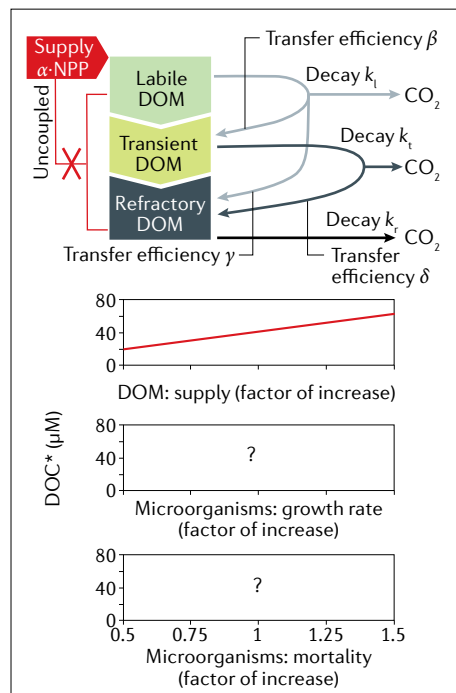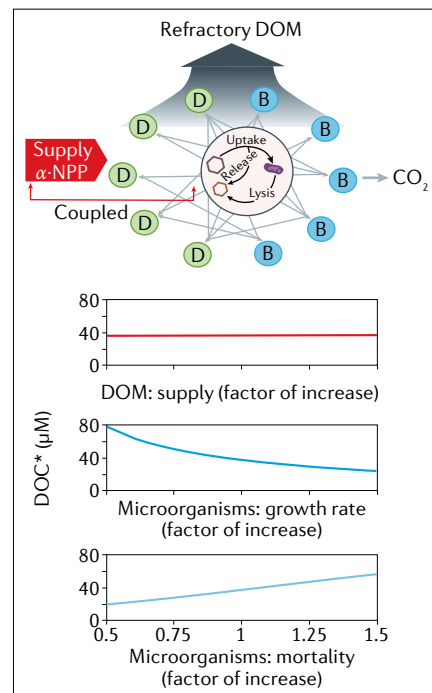

Fig. 4 | Mathematical modelling approaches based on the intrinsic and emergent recalcitrance concepts. The size of the global dissolved organic carbon (DOC) pool is determined by fundamentally different processes, depending on the assumed stabilization mechanism. **a** | According to the intrinsic recalcitrance concept, a decrease in primary production in a future ocean (less supply) might decrease the steady-state concentration DOC* and, therefore, the global DOC pool. The decay rate of dissolved organic matter (DOM) is an intrinsic property of DOM in the model, depending on its decay rate constant and the transfer efficiency between DOM pools. There is no feedback to microbial abundance. **b** | In the emergent recalcitrance concept, there is a direct feedback between supply and decay. DOC* is not sensitive to changes in primary production (NPP) as in panel **a**. Microbial abundance and traits are integral parts of the model. Parameters for the simulations in the lower panels were varied by a factor of 0.5 to 1.5 from their default values (factor of increase on the x-axes) that reproduce the best observations in today's ocean. Transient DOM refers to the semi-labile and semi-refractory fractions together, which have relatively short lifetimes. The numerical simulations performed here are based on published work[1,32,36,111] (Supplementary information). α, β, γ, δ, transfer efficiencies between pools; B, abundance of bacterial species; D, concentration of individual substrates; k_l, k_t, k_r, decay rate constants for the respective reactivity pools.

Box 1 | **Comparison of common model formulations based on the intrinsic and emergent recalcitrance concepts**

Intrinsic recalcitrance
Prescribed reactivity pools of dissolved organic matter (DOM)
In steady state, the rate of change of dissolved organic carbon (DOC) concentration depends on supply (αNPP in our exemplary model formulation[203], for example, from phytoplankton exudation), the first-order degradation rate constant that prescribes the reactivity (in this example, k_l, k_t, k_r decay rate constants for respective reactivity pools) and the efficiency of transfer between the reactivity pools α, β, γ and δ. The steady-state DOC concentration DOC* ($\frac{dDOC}{dt} = 0$) depends on all of these parameters:

$$DOC^* = \alpha NPP \cdot \left(\frac{1}{k_l} + \frac{\beta}{k_t} + \frac{\gamma + \beta\delta}{k_r} \right)$$

Mechanism
The main stability mechanism acts at the molecular level; that is, the intrinsic properties of the molecules define their degradation rates in relation to other compounds. DOM degradation is directed towards higher recalcitrance: labile DOM is converted into transient and then refractory DOM. The reaction rate is controlled by the DOC concentration of the respective reactivity pools and the decay rate that represents their reactivity.

DOM degradation rate
DOM decay is described as a first-order reaction, depending only on the DOC concentration and its decay rate constant, representing its reactivity.

Long-term changes
Production and consumption are decoupled, as there is not a strong feedback between DOM and the microbial community: changes in production and consumption equally influence the size of the global DOM reservoir in steady state (see increasing DOC* for increasing supply in FIG. 4a).

Emergent recalcitrance
Reaction network of microorganisms and compounds
In steady state, the rate of change of DOC concentration emerges from a network of microorganisms and compounds. The rate of change of DOC concentration depends on supply (αNPP in our exemplary model formulation[36]) but also on the abundance of microorganisms that, in turn, grow depending on the availability of their substrate (dynamic feedback). As a result, DOC* depends on microbial properties (such as mortality μ, growth efficiency η, maximum turnover ρ in this example) and DOM molecular diversity (n):

$$B^* = \frac{\eta}{\mu(1-\beta)(1-\eta)} \cdot \alpha NPP$$

$$DOC^* = \frac{\mu}{\eta} \cdot \frac{\kappa}{\rho} \cdot \frac{n}{n_u}$$

where n_u/n (reciprocal of n/n_u in equation above) is the fraction of total DOM compounds that a specific bacterium is able to take up and κ is the half-saturation constant.

Mechanism
The main stability mechanism acts at the community level; that is, the concentration distribution of DOM constituents and the microbial community determine DOM degradation. Stability emerges on the level of the reaction network given that (1) one microorganism can only consume a subset of the DOC compounds present and (2) microbial DOM uptake decreases with decreasing substrate concentration. There is no directionality, as each compound can serve as both product and substrate.

DOM degradation rate
DOM decay is described by a second or higher order reaction, with DOC concentration and microbial community as the 'reactants'.

Long-term changes
Production and consumption are coupled because of the feedback between DOC concentration and the microbial community. Microbial traits have the largest impact on DOC*. The rate of production of fresh DOM influences the global DOM reservoir only temporarily (see stable DOC* for varying supply in FIG. 4b).

evolve within 70 years[101]. The degradation of polymers such as polysaccharides (which feature high levels of chemical complexity[102]) illustrates the abilities of microheterotrophs to degrade DOM, even when it might require hundreds of different enzymes, such as with fucoidan degradation[64]. In the absence of hydrolytic enzymes or other catalysts, polysaccharides (such as fucoidan) are highly recalcitrant, as the intramolecular bonds are stable for millions of years in aqueous solution[103,104]. In addition, mechanisms have evolved that enable bacteria to break down larger polymers that cannot be taken up from solution directly; rapid binding and hydrolysis of polymers occur on the outer membrane, followed by the transport of fragments into the periplasm[105].

The implementation of the intrinsic recalcitrance concept into numerical models relies on formation and decay rates that are derived from present-day observations integrated over ocean circulation timescales[5]. Although these models can reliably reproduce the global distribution pattern of DOM in the contemporary ocean, the predictive power for future climate scenarios is limited because the formation and degradation kinetics of refractory DOM might change in unknown directions in a future ocean.

Implications in the context of global change.
The carbon sequestration potential via the microbial formation of the long-lived fraction of recalcitrant DOM (>100 years) was estimated at 0.2 petagrams of carbon per year in today's ocean[100]. This potential rivals transport of carbon into the deep ocean (>2,000 m depth) via sinking biogenic debris (0.4 petagrams of carbon per year)[100]. The intrinsic reactivity concept is intriguing because it implies a positive feedback between long-term carbon sequestration and marine primary production[106] and, therefore, climate geoengineering potential[107,108]. However, the formation rate of recalcitrant DOM in a future ocean and the magnitude and direction of potential changes of the marine DOM inventory are unknown[100].

General trends on the response of the global DOM pool to climate-related changes can be deduced from numerical models, which inherently simplify natural processes by limiting the number of degrees of freedom (FIG. 4). Models that implement the classical intrinsic reactivity concept do not include direct feedback mechanisms between microbial consumers and DOM reactivity pools. Once produced by microorganisms, recalcitrant DOM fractions decay following prescribed first-order kinetics (FIG. 4a). In these models, which typically assume globally uniform degradation rate constants[39,109–111], the DOM pool size depends on external supply and is linked to primary productivity. New nutrients exert fundamental control on DOM accumulation in the surface ocean[41,42],

as well as the composition of the microbial community[112,113].

Nutrient dynamics and microbial communities will both likely change in a warmer, less basic, less oxygenated and more stratified ocean[114,115]. If the formation of refractory DOM is related to the surface accumulation of DOM, major changes in the global DOM pool can be expected over the next century. For instance, stronger water-column stratification owing to ocean warming would reduce the input of nutrients to the surface, in turn reducing primary production and, therefore, supply of substrate to microheterotrophs, potentially resulting in less formation of refractory DOM[100]. The contrary might be the case if the refractory DOM fraction is primarily formed by abiotic photochemical reactions at the sea surface, because of the expectedly higher transparency of surface waters in the future[20]. However, a slowdown of the deep thermohaline circulation in the ocean would potentially decrease the rate of photochemical decomposition of deep-sea DOM, although it would require hundreds of years to take effect.

Emergent recalcitrance

According to the emergent recalcitrance concept, individual DOM constituents are continuously reworked. They are produced, modified and consumed within complex ecological networks encompassing all trophic levels, including phytoplankton, bacteria, viruses and grazers. The concept considers that marine microorganisms and non-living organic matter form an inseparable liaison[116] and organic molecules are integral to ecological systems[116,117]. In this concept, recalcitrance is not an intrinsic property of individual DOM constituents but emerges as a bulk property on an ecosystems level[35,36], like the proposed mechanisms causing persistence of organic matter in soils[4].

Models that implement the emergent recalcitrance concept do not prescribe reactivities to DOM fractions a priori[35,36] (FIG. 4b). Instead, DOM is considered a mixture of individual compounds, while the microbial community consists of individual taxonomic units. Microorganisms and substrate compounds interact within the resulting degradation network[36] (FIG. 4b). All compounds are continuously produced and consumed, and, over the long term, their concentration is controlled by this dynamic equilibrium[36].

Lines of evidence. There are three major lines of evidence in support of this concept. First, numerical models confirm

the consistency of the concept. Recently published mechanistic models showed that fundamental microbiological principles can explain the long-term persistence of DOM[36,97] (FIG. 4b). Intrinsic recalcitrance is not required to explain the longevity of DOM in models composed of a diverse network of microorganism–substrate interactions[36]. Microbial growth ceases when the concentration-dependent uptake of DOM balances the concentration-independent microbial carbon loss via mortality or maintenance, resulting in an equilibrium level of DOM[36] (FIG. 4b). These model experiments reproduce all major properties of DOM put forward as evidence for the existence of the intrinsic recalcitrance of microorganism-derived DOM[36]. For instance, in these models, an initial pulse of a single compound (for example, glucose) results in the accumulation of >15% of the initial DOM addition as a molecularly diverse mixture[36], and this residual is consumed by microheterotrophs if concentrated[36]. Deep-sea DOM concentrations of 30–40 μM carbon, as well as observed depth profiles (FIG. 1), are explained in the model by the physiology of microorganisms[35]. Furthermore, despite being labile, DOM compounds reach radiocarbon ages between modern and several thousand years in the model[36].

Second, observations confirm that natural microbial communities use deep-sea DOM as growth substrate[118,119]. Earth's crust below the seafloor is a natural incubator, where deep-sea DOM is entrapped and consumed by microheterotrophs. These microbial communities are sustained mainly by the oxidation of deep-sea DOM, and, after residing 2,000 years in the porous crust, DOM concentration has dropped by 50% to 20 μM carbon[118]. Furthermore, experimental concentration of deep DOM results in microbial growth, supporting that not all DOM is intrinsically recalcitrant[119]. DOM consumed in these experiments was derived from the deep sea and molecularly highly diverse, indicating that a broad range of DOM constituents was consumed at an enhanced rate if concentrated[119].

Third, the radiocarbon age, reactivity and structure relationships discussed here are consistent with the emergent recalcitrance concept. The growth rate is proportional to the supply rate in steady state. Thus, compounds produced at a high rate encounter microbial cells more frequently than rarely produced compounds, increasing the likelihood of uptake. Consequently, the radiocarbon age of individual compounds is not necessarily determined by intrinsic

reactivity but by the rate of supply from primary production when the radiocarbon clock is set to zero[36]. Indeed, compound groups that are enriched in phytoplankton blooms (hydrolysable carbohydrates, high-molecular-mass compounds, colloidal matter) have younger radiocarbon ages even in the deep sea than those that become enriched during microbial degradation (hydrophobic, small and chemically resistant compounds)[56,60,92].

Microheterotrophs living in water entrapped in Earth's crust preferentially consume younger rather than older DOM compounds[118], which might have structural reasons (intrinsic recalcitrance). However, this observation can also be explained by the time a compound has spent in the degradation network, because degradation slows with time elapsed after its supply from primary production (emergent recalcitrance)[36]. The observed incorporation of aged compounds into bacterial biomass in the surface ocean[98] is also consistent with the concept, because molecules of a new photosynthetic product, such as glucose, mixes with old (glucose) molecules, such that they are more concentrated and then degraded at a higher rate[36].

DOM stabilization mechanism. In the degradation network, the more distant an organism is from primary production (more steps away from supply in FIG. 4b), the more it is subjected to a molecularly diverse and dilute substrate pool. The passage of biogenic sinking particles at depth and transient algal blooms at the sea surface expose many of the organisms living in the water column to irregular 'feast or famine' conditions[120]. Therefore, organic matter degradation in the water column does not follow a predefined timeline but occurs within complex and nested interactive networks. To understand DOM decomposition under such conditions, it is important to consider that the consumption rate of a substrate slows as its concentration declines[119,121,122].

For the simplest case of one taxon growing on a set of suitable substrates, growth rates can be approximated by the Monod equation[123], in which growth and substrate consumption rates are linearly related[123]. Although the Monod curve dominates models of microbial growth, knowledge about growth at very low substrate concentrations is scarce[124] (FIG. 5). In culture, bacteria respond to concentrations of a single growth substrate as low as 1 nM (REFS[125,126]). Some organisms grow on mixtures containing

up to 45 different organic compounds[127]. If these numbers from culture experiments are applied to the marine environment, bacteria living on substrate mixtures might be able to survive at an individual substrate concentration of >20 pM (1 nM
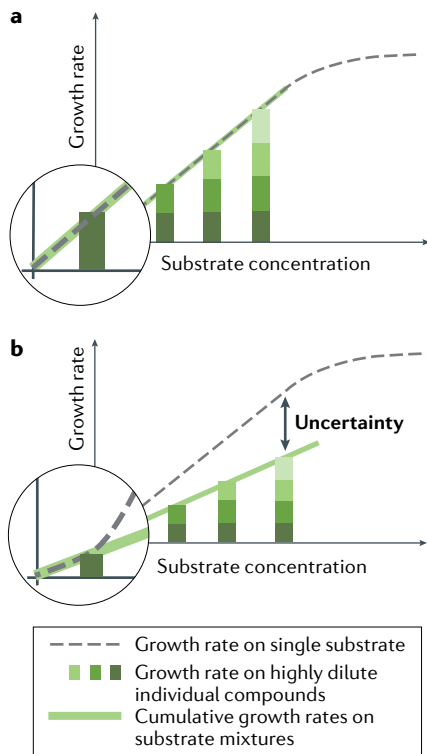

Fig. 5 | **The challenge of upscaling kinetic constants at low substrate concentrations.** The classical Monod equation for microbial growth on a substrate is constrained by two parameters: the maximum growth rate (saturation) at high substrate concentration and the substrate affinity (half-saturation constant)[123]. Together, these parameters define the slope of the growth curve. However, at very low substrate concentrations, power gain from a chemical reaction approaches the power investment for cell maintenance, with growth further reduced or halted[138–140]. This deviation from Monod kinetics leads to uncertainties when scaling up from a single substrate to a diverse mixture of substrate compounds. **a** | A theoretical, fully linear case for growth at low substrate concentrations. The cumulative growth rate on a mixture (green bars) equals the growth rate on a single substrate (grey dashed line) when the bulk concentration of the mixture equals that of the single substrate. **b** | The theoretical case where nonlinearity of the growth rate at very low concentrations is considered. Deviation from the linear response at low concentrations leads to an overall lower growth rate on a diverse mixture than a single substrate at the same bulk concentration. In this theoretical case, affinity and maximum growth rate are set equal for all substrate compounds to illustrate the overall concept. The same principle applies to mixtures with varying individual, substrate-specific kinetic constants.

minimum substrate concentration divided by 45 compounds).

The concentrations of individual compounds in the highly diverse marine DOM mixture are unknown. As a conservative approximation of an upper limit, one can divide dissolved organic carbon concentrations in the deep sea (>34 μM) by the estimated minimum number of compounds in DOM (>600,000) and the average carbon number in a DOM molecule (~20)[128]. According to this estimate, the concentration of individual DOM compounds would be <3 pM. Because DOM is possibly composed of millions of constituents, their actual concentration might be orders of magnitude lower. These considerations suggest that the concentration of some DOM constituents is too low to induce microbial growth and that they escape biodegradation owing to dilution.

Yet, cells in the natural environment subsist at energy fluxes five orders of magnitude lower[129] than in culture[130]. In the subseafloor, individual cells survive by oxidizing as little as about one organic carbon atom per second[131,132]. In the presence of oxygen, cells retain their metabolic potential under extremely low energy conditions in deep marine sediments even up to 100 million years[133]. Bacterial cells have developed two strategies to survive under such energy-limiting conditions[134]. The first is the simultaneous expression of many catabolic enzyme systems[135], which enables organisms to thrive on several different substrate compounds, alleviating the limitation of low substrate concentration. In the water column and in sediments, marine microbial communities transcribe many genes encoding transporter proteins simultaneously, despite presumably very low concentrations of individual substrates[136,137]. This generalist strategy requires diverse metabolic investment and is energetically costly. The second strategy is to minimize requirements for general cell and enzyme maintenance, for example, through replacement of damaged proteins instead of producing new cells by division[138]. At some point, at very low substrate concentrations, thermodynamic and physiological constraints are unbreachable[138–140].

To summarize, given the number of potential substrate molecules residing in DOM, the multitude of organisms that exist everywhere in the ocean[141] and the enzymatic potential in the ocean (FIG. 6), the size of the resulting reaction network is enormous and potentially explains the stabilization and persistence of marine

DOM. The microheterotrophs able to degrade a molecule decrease in abundance together with the availability of their substrates[122], thereby slowing decay of the overall DOM pool[36]. With the rate slowing as concentrations decline, a fraction of DOM can persist over thousands of years[36], while the diversity of DOM molecules is maintained.

Limitations. The emergent recalcitrance concept provides a plausible explanation for the longevity and main properties of DOM in the ocean. The numerical models are parameterized by the state of knowledge on microbial physiology, but this knowledge is almost entirely derived from culture experiments or observations at the sea surface. There is too little understanding of physiological adaptation strategies of microorganisms at high pressure and extremely low substrate concentrations, even though these could have important impacts on DOM processing. For example, pressure exhibits a major influence on enzyme function[142]. Deep-sea microheterotrophs might possess enzymes with high levels of substrate promiscuity, which could reduce the metabolic cost per substrate used. Filling these gaps of knowledge would help to parameterize physiological components in models[35,36,97] to deep-sea conditions.

Additionally, aggregation of macromolecules into colloids, gels and particles, and sorption of small molecules onto biofilms and cell surface layers[143–145] create microhotspots[120]. In such features, otherwise dilute molecules become concentrated[143,145], potentially enhancing their microbial degradation. Interestingly, a surface-attached lifestyle plays a greater role in the deep sea than at the sea surface, and microbial degradation in the substrate-limited deep ocean appears dominated by particle-associated processes[146]. Deep-ocean microheterotrophs are also known for their extracellular polymeric substances, which can contribute to aggregation[144,147,148]. These microfeatures might be crucial for DOM turnover but are not yet considered in global models that address DOM turnover. As there is a lack of fundamental knowledge on microbial life in the deep sea, and how organisms and communities will respond to global changes, current models have little predictive power for future scenarios.

Implications in the context of global change. Continuous microbial decomposition entails a strong coupling between DOM sources, transformation and degradation

by microheterotrophs. One of the major differences to the intrinsic reactivity concept is the existence in emergent recalcitrance of a feedback mechanism between DOM supply and degradation. General trends on the response of the global DOM pool to climate-related changes can be deduced from numerical models (FIG. 4). Following the emergent recalcitrance concept, an increased DOM supply would increase carbon-limited microbial DOM turnover and, thus, ultimately increase DOM degradation[36], thereby stabilizing the size of the DOM pool. Therefore, changes in primary production would not affect the global DOM pool to a significant degree but instead change bacterial biomass in the ocean, which is of minor relevance in terms of carbon storage[36]. It is important to note that this feedback mechanism refers to the long-term equilibrium state and does not include temporal coupling between DOM supply and concentration as, for example, during and after bloom events[149,150]. The proposed feedback mechanisms imply that any geoengineering strategy aimed at increasing heterotrophic microbial productivity to enhance carbon storage would have regional but likely no long-term effect.

According to models including microbial–DOM feedbacks, the size of the global DOM pool depends largely on the prevailing microbial community[35], in turn controlled by environmental conditions such as temperature, macronutrients and oxygen concentration[151,152], and community interactions[153]. Microbial communities can have multiple steady states[154]. Large-scale environmental perturbations might force marine microbial communities into different steady states at a global scale. Long-lasting changes of the global DOM pool are a possible consequence. The capability of microheterotrophs to consume diverse substrates is crucial in this context. For instance, if the average microbial cell would be able to consume twice as many different substrate compounds as today, the global DOM pool might shrink to half its size within decades[35,36] (n/n_u in BOX 1). This assessment is based on theoretical considerations and numerical modelling; the true magnitude and direction of possible changes are unknown.

Causes of molecular diversity
The very high level of molecular diversity is a key property of DOM and the foundation of the emergent recalcitrant concept, but the causes of diversity are not well understood. Simple, monomeric substrates (such as glucose or glutamate) can be consumed
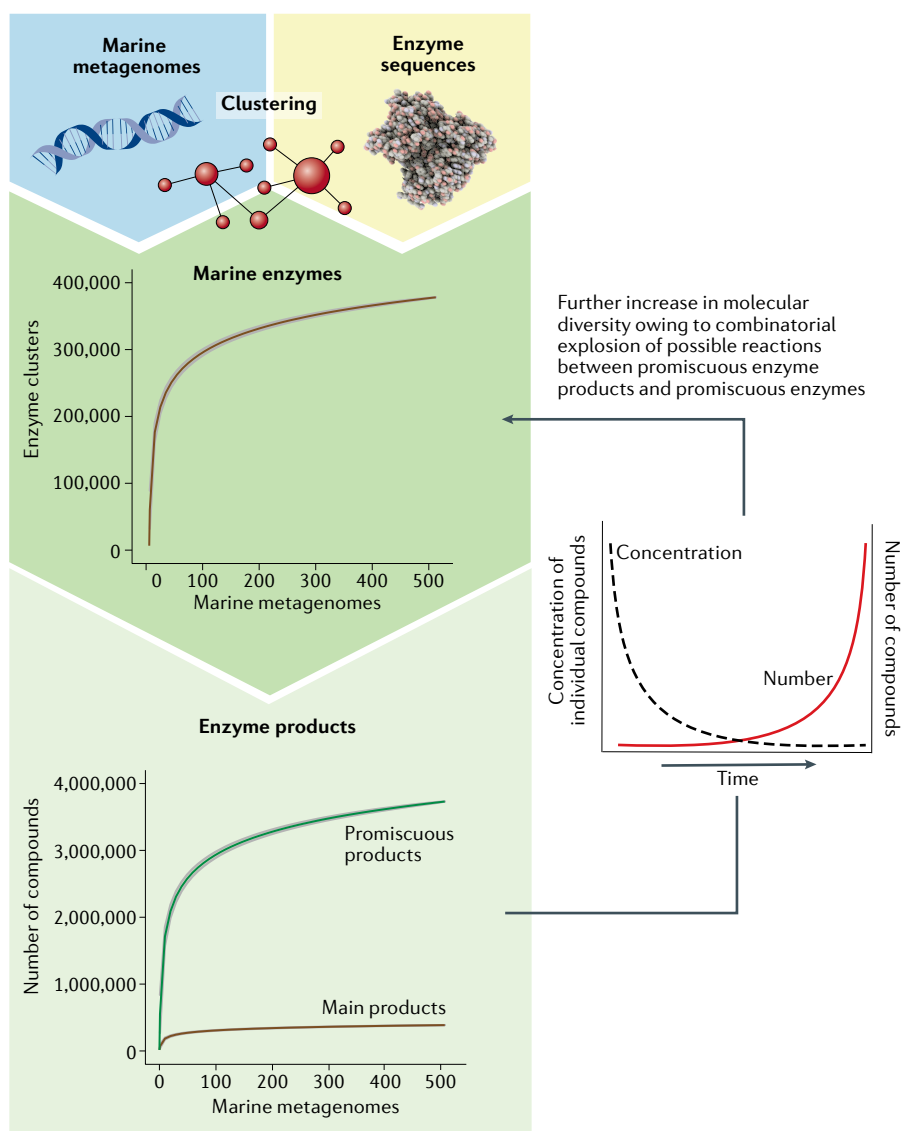

Fig. 6 | **Molecular diversification through enzymatic reactions in the ocean.** To assess the potential of enzymatic reactions in marine organisms, 517 published marine metagenomes[167] were searched for 6,263,764 known enzymes (KEGG[200] and CAZy[201]) via a microbial gene cluster approach[167] (Supplementary information). From this integration, we retrieved 410,199 enzyme gene clusters. The rate of accumulation of these marine enzyme clusters as a function of the number of marine metagenomes is shown (upper diagram). The accumulation rate was calculated using 1,000 permutations and the grey areas around the curves represent the 95% confidence intervals. The curve does not reach the maximum, indicating that the marine enzyme space is highly diverse and still not fully characterized. For each enzyme, we assume one main product (lower diagram, red line) and, conservatively, ten promiscuous products[159] (lower diagram, green line). However, even single amino acid exchanges at crucial positions, which escape bioinformatic detection, can alter an enzyme's specificity or activity[202]. Thus, our analysis is conservative and likely underestimates the number of different molecules produced in the marine environment. As promiscuous products are again subject to enzymatic turnover, many more enzymatic products are to be expected, owing to the mathematical combinatorial explosion of possible reactions between enzymes and substrate compounds (right panel).

by natural marine microbial communities within days to almost undetectable traces[31]. Simultaneously, the organisms release a suite of newly produced dissolved organic molecules to the surrounding water[31]. Only a minor fraction of this newly biosynthesized DOM contains analytically recognizable biomolecules, such as

peptides or carbohydrates[31], and the DOM constitutes a complex mixture of thousands of compounds[106]. These complex molecular mixtures degrade very slowly or not at all over years in culture[31,106,155].

The substantial metabolic potential of marine bacteria was illustrated when two single strains of the *Roseobacter* group,

a ubiquitous marine bacterium, were grown in pure culture on simple organic compounds such as glucose or acetate as sole carbon sources[156]. Within a few days, the microorganisms converted the simple substrates into a myriad of compounds, similar in complexity to marine DOM. While the organisms were growing, more than 25,000 distinct compounds were released to the seawater medium even after substrate depletion[156]. Considering the simplicity of the experiment, where grazers, viruses, complex substrates and ecological interactions were excluded, the degree of molecular diversity of the exometabolome (the collection of extracellular metabolites) was unexpected. Out of the 25,000 products, less than 1% (<100 compounds) matched genome-predicted metabolites[157].

Based on these observations, we hypothesize that microbial processes are key to sustaining the large molecular diversity of DOM. In this context, we highlight enzymatic promiscuity that inevitably leads to unforeseen molecular reactions and diversification of DOM. Enzymes are capable of catalysing physiologically irrelevant, promiscuous reactions that are different to the reactions that they have evolved to catalyse[158–161]. For instance, the enzyme RuBisCO mediates carbon fixation in most autotrophs by forming a C–C bond between its canonical substrates CO_2 and ribulose-1,5-bisphosphate. However, RuBisCO exhibits promiscuous activity with O_2 and ribulose-1,5-bisphosphate, leading to oxygenation instead of carbon fixation[162]. This product of RuBisCO's promiscuity[163] has an estimated flux of 1 petagram of carbon per year in the ocean and can be assimilated via the recently discovered β-hydroxyaspartate pathway, found in 1.5% of bacterial cells during a spring bloom[163].

The number of promiscuous reactions available in nature is considerable[164]. Enzymes likely catalyse at least ten promiscuous reactions, albeit mostly at a level of activity too low to elicit positive selection for new pathways that could use the promiscuity products[164]. A similar number of promiscuous affinities has been observed in natural seawater for extracellular peptidases[165]. Side reactions performed by enzymes that catalyse the microbial loop could contribute to the diversification of DOM and its persistence in the environment. The refractory DOM pool is sustained by a minor fraction of the annual primary production; thus, small error rates during enzymatic processing would suffice to sustain the refractory DOM pool in the ocean.

The different microorganisms and their enzymes in the ocean source an immense diversity of molecular intermediates and products[166]. To assess the potential of enzymatic reactions in the ocean, we explored the presence of enzyme sequences in a set of public marine metagenome databases (FIG. 6). We integrated more than 6,200,000 enzyme sequences into a comprehensive dataset of metagenomic and genomic microbial gene clusters[167]. From this integration, we retrieved 410,199 enzyme gene clusters (see Supplementary information for methods). This estimate is conservative because even single amino acid exchanges at crucial positions can alter enzymes' specificity or activity, thereby escaping bioinformatic detection. In addition, global metagenome surveys have not yet covered all marine habitats, the viral metagenome remains largely unknown and not all enzymes present on Earth are identified and registered in bioinformatic databases. Considering that individual enzymes likely catalyse at least ten promiscuous reactions as a conservative estimate[164], marine organisms potentially produce more than 4,000,000 different products (FIG. 6).

This estimate does not consider that promiscuous products are again subject to enzymatic modification. Downstream processing results mathematically in a combinatorial explosion of possible reactions, and orders of magnitudes more enzymatic products are to be expected. It could be argued, although, that combinatorial explosion is unlikely because cells limit side reactions by manufacturing metabolites on demand and compartmentalization[64] — tight networks and compartmentalization limit interactions between enzymes and substrates in living cells[168]. However, cell death leads to encounters of formerly separated molecules, enzymes and pathways unforeseen in a living cell. The main sources of microbial mortality in the ocean are viral lysis[169–171] and grazing by protists[171,172]. The digestive organs of grazers are active bioreactors where microbial biomass is modified through manifold enzymatic reaction. Viral lysis accelerates the transformation of microbial into dissolved matter[169,170] and leads to a rapid dilution of cellular molecules and reduction of encounter rates in the environment. With this background, a maximization of molecular diversity during organic matter turnover in the ocean seems inevitable.

In addition to enzymatic reactions, abiotic or enzymatically catalysed radical reactions that occur in the ocean could contribute to molecular diversification. Superoxide and other reactive oxygen species are continuously produced at the sea surface and in rain owing to photochemical reactions[173] and in marine sediments at redox interfaces[174]. In addition, all aerobic organisms produce reactive oxygen species as a metabolic by-product, and, in the dark ocean, taxonomically and ecologically diverse microheterotrophs are a vast source of superoxide[175,176]. These radicals react in largely chaotic fashion with organic molecules to create diverse suites of degradation products in the environment[177,178]. DOM is also molecularly altered by direct photochemical reactions at the sea surface[179–181], abiotic sulfurization in sediments[182] and by geothermal heat[183]. Despite globally low reaction rates, some of these abiotic processes might cumulate to global-scale effects[46,76,182,183]. On geological time spans, racemization and other abiotic reactions towards a chemical equilibrium state occur, but these processes are slow and mostly negligible in the presence of active microorganisms[86,184,185].

Summary and challenges
Despite substantial progress over the past few decades, the mechanisms underlying the longevity of DOM remain elusive. A long-standing paradigm proposes that intrinsic molecular properties rule long-term turnover kinetics of DOM. A novel, alternative concept recognizes the complexity of interactions between microbial communities and DOM, both of which are highly diverse in terms of composition and function. This new concept proposes that DOM persistence emerges as a bulk property from these interactions, even though all DOM compounds are potentially biodegradable. Both intrinsic and emergent recalcitrance are consistent with observations in today's ocean, but they imply very different responses of the global ocean DOM reservoir to climate-related changes.

It is time for new research directions that integrate current knowledge and reconcile the contrasting concepts that are put forward to explain the longevity of DOM. Which of the two main stabilization mechanisms prevails is unknown. It is important to recognize the complexity of the system, which we describe here as the ecology of molecules: individual compounds of the non-living organic matter pool and individual organisms interact within an environmental context. The nature of these ecological interactions is largely driven by intrinsic traits of the individual living and non-living players. From these interactions,

system properties emerge that the individual players do not have on their own. Current models that are based only on the intrinsic recalcitrance concept ignore the complexity of the system and the interactive network between organisms and molecules, while models built on the emergent recalcitrance concept largely ignore molecular properties and individual microbial traits.

With emerging systems biology and systems ecology approaches, ecological interactions in plankton blooms are beginning to be understood on a truly mechanistic level from genes to ecosystems[96,152]. However, the enormous molecular diversity of microbial exudates and the highly complex interactive network between organic compounds and microbial cells are not currently considered in these approaches. The ecological network that arises through the interplay of the 'rare biosphere'[141] and the 'rare chemosphere'[116] is largely unappreciated. Many compounds are produced and turned over at very low rates, which might be of little relevance to understand bloom situations, but these rare processes likely control the size of the global DOM pool. To fully appreciate the ecology of molecules, new approaches are needed to conceptualize ecological interaction networks of such diversity and dilution, as classic concepts from theoretical ecology might not be applicable. Notions from statistical physics and complex systems theory[37] could aid in identifying system properties that emerge from the diverse ecological interactions. These emerging properties, such as DOM persistence, should be the target of experimental incubation and field studies.

Advances in the field are now largely driven by long-term collaborative efforts and multidisciplinary approaches where chemists, biologists, oceanographers and mathematicians work together. Much will be gained if the large scientific communities who study the turnover of non-living organic matter residing in soils, the marine subsurface and in the water column collaborate more closely, as much of the knowledge gained in any of these systems is universal. On the way towards a mechanistic ecology of molecules understanding, there are major research challenges, which we highlight next.

Assessing molecular diversity of DOM.
Molecular diversity is not a traditional analytical target in geochemistry but a key feature of marine DOM from which novel bulk properties emerge. It has been assessed mainly on a molecular formula level via ultrahigh-resolution mass spectrometry[67], thus not capturing all diversity characteristics that are relevant. Emerging high-field nuclear magnetic resonance[186], multidimensional chromatography[187] and enzymatic and immunoassay[63] approaches must be further developed for the assessment of DOM structural diversity. Advanced chemometric data tools will be crucial for the integration of complex molecular data[188,189]. One goal should be the establishment of molecular fingerprints as proxies for molecular diversity with the help of high-throughput and high-resolution analytical techniques to assess large sample sets with reduced analytical effort.

Promiscuity of enzymes.
Field observations and experiments usually do not consider rare processes and extremely low metabolic rates that cumulate to global-scale effects. Rare promiscuous enzymatic reactions in living cells and after death should be the focus of experimental research. Ultimately, promiscuous enzyme products should be included in metabolic databases and bioinformatic approaches for a mechanistic understanding of the molecular diversification of DOM or the formation of recalcitrant structures. Furthermore, promiscuity is an intrinsic feature of enzymes allowing evolution to manifest new reactions. The number of proteins on Earth, and, consequently, the molecular diversity of reaction products, is constantly expanding via evolution[190]. The linkage between enzymatic substrate diversification and microbial evolution should be explored in culture studies and eventually implemented in ecological and biogeochemical models.

Abiotic processes.
Knowledge is scarce as to whether abiotic reactions, for instance with reactive oxygen species, contribute to the molecular diversification[178] or the formation and destruction of specific refractory structures[85]. Like enzymatic side reactions, slow abiotic processes could cumulate to global-scale effects[46,76]. The molecular diversification of simple substrates through abiotic reactions and the bioavailability of the reaction products to marine organisms should be further explored in joint microbial and chemical experiments. Potential hotspots of abiotic reactions at the transition zones between sediment, water column, suspended particles and atmosphere should be the focus of future studies.

Physiological adaptation to deep-sea conditions.
Improving the quantitative understanding of physiological adaptation strategies of microorganisms to high pressure and extremely low substrate concentrations at the energetic limit of life is crucial to improve the parameterization of numerical models (FIG. 4). It must also be considered that microorganisms detaching from sinking particles are exposed to 'feast to famine and back' conditions. Long-term culture experiments with marine organisms are needed to study physiological adaptation strategies. The porous crust below the seafloor could serve as a natural laboratory for incubations lasting millennia[118]. Genomic and transcriptomic studies of single cells[118,191] combined with in situ or pressure-retaining incubation experiments[192,193] are promising approaches to understand adaptation under natural conditions.

Environmental heterogeneity.
Environmental conditions vary on all scales from micrometres to kilometres and within minutes to seasons[16,120,194]. For instance, the nutrient-rich microenvironments next to particles or living cells are hotspots of microbial activity in the abyssal ocean[120]. The microbiome of marine sponges is another example of such a hotspot[195]. Sorption onto surfaces of these microhotspots could yield molecules too dilute in the dissolved phase for microbial degradation degradable again. Also poorly understood is the effect of large-scale biogeographical patterns on DOM turnover. We recommend exploring patterns of molecular diversity with help of the above-mentioned molecular signatures through space and time as a regular parameter in large-scale oceanic surveys, but also in poorly explored microenvironments.

The challenge of upscaling.
Understanding global effects requires upscaling, thereby bridging many orders of magnitude in space and time (FIG. 2). The lack of process understanding on the levels described above introduces bias that propagates unpredictably when extrapolated to the global scale because of nonlinear processes and positive or negative feedbacks. Global model development in the sense of the ecology of molecules should address interactions on the community and ecosystem scale on all trophic levels under consideration of environmental conditions. The implementation of such ecological models into global biogeochemical ocean models is in its infancy. Global models that predict phytoplankton diversity[196] and a new modelling framework that relates DOM accumulation to ecological dynamics[97] are promising examples of first steps in this direction. State-of-the-art

Earth system models have a nested and flexible model infrastructure, allowing the successive integration of new ecological and physiological concepts[196–198]. Further expanding the basic 'nutrient–phytoplankton–zooplankton–detritus' structure of biogeochemical models with an explicit representation of microbial and molecular DOM diversity and ecological interactions would be an essential step to successively integrate the ecology of molecules concept in global models.

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Author contributions
T.D. chaired the writing of the article. S.T.L. performed the numerical model and C.V. the bioinformatics computations. T.D. measured the ultrahigh-resolution mass spectra shown in Fig. 1. Otherwise, the authors contributed equally to all aspects of the article.

Competing interests
The authors declare no competing interests.

Peer review information
Nature Reviews Earth & Environment thanks Andrew Steen and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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Supplementary information
The online version contains supplementary material available at https://doi.org/10.1038/s43017-021-00183-7.